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DANIEL STEWART THOMAS ROBERTS

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GROWTH, NODULATION AND NITROGEN FIXATION OF ALFALFA

(*Medicago sativa* L.) AND RED CLOVER (*Trifolium pratense* L.)

AS INFLUENCED BY SOIL ACIDITY AND LIMING

by

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B.A., Sacred Heart University, 1969

M.A., Connecticut College, 1974

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This thesis has been examined and approved.

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ABSTRACT

GROWTH, NODULATION, AND NITROGEN FIXATION OF ALFALFA
(Medicago sativa L.) AND RED CLOVER (Trifolium pratense L.)
AS INFLUENCED BY SOIL ACIDITY AND LIMING

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DANIEL STEWART THOMAS ROBERTS
University of New Hampshire, September 1980

A series of field, greenhouse and laboratory experiments were conducted to examine the influence of soil acidity and liming on the growth of Rhizobium and the nodulation, nitrogen fixation, and yield of alfalfa and red clover.

In the greenhouse, from soil pH 5.0 to 5.6, nitrogen fixation was shown to be more correlated to Al saturation than pH or available P. Once Al saturation was reduced to less than 5 percent by liming, the application of P was more beneficial to yield and nodulation than further application of lime. Lime pelleted alfalfa seed sown at a soil pH of 5.0 increased nodule weight and number but did not improve nitrogen fixation or yield. Increasing the soil pH by soil-incorporation of lime eliminated the advantage of pelleting. The growth response of red clover to liming was less than for alfalfa. Work with ^{32}P in hydroponics showed that red clover was capable of mobilizing P under acidic conditions in the presence of Al to a greater

degree than alfalfa. The ratio of Al/P in the nodules was significantly less in red clover than alfalfa as measured by electron dispersive analysis by X-ray.

Using an aeroponic culture, nitrogen fixation by red clover was more tolerant than alfalfa to Al stress at pH 5.0. The addition of nitrogen, however, reduced nitrogen fixation in both legumes by over 90 percent after four weeks. Nitrate reductase activity in red clover was shown to be four to five times that in alfalfa when the plants were grown at pH 5.6. The addition of fertilizer nitrogen to the soil increased nitrate reductase activity in both legumes with a compensatory decrease in nitrogen fixation such that yields were not affected.

The growth of *Rhizobium meliloti* in acidic media was shown to be influenced by Al. At pH 5.0, the addition of the element severely reduced growth in the three strains tested. At pH 5.5, there was an increase in growth lag, but the strains demonstrated a differential adaptation to the toxicity. In order to test the effectiveness of these strains in soil, three alfalfa varieties were inoculated and seeded into sterile soil at pH 5.0, 5.5, and 6.0. No single combination of strain-variety gave the highest yield, nodule number, or nitrogen fixation for all three soil pH's.

Two field experiments were established to study the influence of banded/broadcast lime and phosphorus on the growth of alfalfa and red clover established by minimum tillage in an acidic soil. In the Harmony Hill experiment, lime rate from 4.6 to 13.8 t/ha had no influence in the soil pH of the top 5 cm of the profile. At greater depths, the higher lime rates raised pH and significantly increased yields. Banded lime did not improve yields, percent legume, or stand persistence. One year after establishment, red clover averaged less than 25 percent of the stand; inspection indicated severe internal breakdown and secondary infection by *Fusarium solani*. The effects of low P and high Al saturation in the subsoil on alfalfa stand persistance is not known.

In the Demerit experiment, hydrated lime increased the soil pH from 5.6 to over 6.5 in the top 5 cm of the soil one month after liming but actually reduced yield and nodulation of the legume. The application of broadcast P decreased the Al saturation in the unlimed plots in the top 5 cm of the soil to levels comparable to the dolomitic and hydrated lime plots. The yields of alfalfa in the unlimed plots equaled those in the limed plots when both sites received a single addition of banded monoammonium phosphate. These higher yields in the banded plots occurred in the first spring harvest following the August 1978 seeding and may have resulted from the presence of both N and P in the band.

Results from the two field experiments indicated that good yields of alfalfa can be achieved in the year following establishment if the acidity is reduced in the top 10 cm of the soil profile and adequate P is present; longevity of the stand remains undetermined.

INTRODUCTION

The importance of alfalfa as a forage legume in New Hampshire has increased greatly in the past three decades. The acreage of hay in the state has decreased by 74 percent since 1950 while alfalfa has increased by 37 percent over the same period. Compared to red clover, alfalfa is more persistent, drought resistant, higher yielding, and has a higher protein and calcium content. The sensitivity to soil acidity does present problems in growing alfalfa in New Hampshire where the pH of virgin soil normally ranges from 4.5 to 5.5.

Under acidic conditions, exchangeable aluminum levels increase to toxic levels and the macronutrients calcium, magnesium and phosphorus decrease in the soil. The effects of these soil processes on legumes varies with species,but in general aluminum interferes in mitotic division in root cells inhibiting lateral and root hair development and prevents full utilization of phosphorus for metabolism. The low levels of calcium reduce nodulation and inadequate phosphorus inhibits dry matter accumulation.

Liming usually alleviates the stress of soil acidity on alfalfa by increasing pH, providing calcium and magnesium and reducing aluminum saturation in the soil. The influence of lime on the plant varies with soil type, liming material, initial pH and plant species. With legumes, the influence of acidity and liming must be considered for the host plant, the specific *Rhizobium* and the symbiotic relationship.

The object of this research was to examine the influence of soil acidity and liming on Rhizobium growth, nodulation, nitrogen fixation, and yield of alfalfa and red clover using field, greenhouse and laboratory experiments.

I. REVIEW OF THE LITERATURE

Historical Perspective

An abundance of nutritive forage is of prime importance for successful livestock production. In this respect, New England was strikingly deficient when first settled. The Indians kept no herbivorous domestic animals and hence had developed no forage plants (Bidwell and Falconer, 1925). Consequently, the hay and pasture plants important to livestock such as timothy, Kentucky blue grass, red clover, white clover and alfalfa were imported from Europe (Bolton, 1962).

Because the native grasses (wild rye and broom straw) were not nutritious enough for cattle, English grasses were imported to New England in the early 1600s. In 1665, bluegrass, red clover, and white clover were seeded in Long Island, New York, and Rhode Island (Flint, 1860). In Pennsylvania and New Jersey, local fields were covered by clover in the 1680s. William Penn wrote about a successful seeding of red clover in Chester County, Pennsylvania, in 1685 (Bidwell and Flaconer, 1925). Occasional reports were published of red clover growing in New York prior to the Revolutionary War. Red clover and timothy were seeded in Portsmouth, New Hampshire, as early as 1700 (Piper, 1925). In the early 1800s, red clover and timothy mixtures covered fields in New Hampshire, Maine, Vermont, and Connecticut. In 1850, Massachusetts had 380,000 acres planted to red clover and timothy mixtures. In the late 1800s, New York led the eastern states with a production of hay in excess of three million tons (Bidwell and Falconer, 1925).

Unlike red clover, the history of alfalfa in the eastern states and especially in New Hampshire is sketchy until 1900. There are brief reports of the legume being grown in Georgia in 1736, North Carolina in 1739 and in New York in 1791 (Bolton et al., 1972). Only on certain calcareous soils in New York was the growing of alfalfa successful. Bidwell and Falconer (1925) mention alfalfa being grown in Pennsylvania in experimental plots in 1820;and in New Hampshire, the legume failed to establish in 1887 due to a severe winter (New Hampshire Experimental Station Report, 1890). In 1908 and 1909, alfalfa was sent out by the New Hampshire Experiment Station to 35 farmers in 18 towns (Taylor, 1910). By 1910, only 20 percent of the stands survived. Taylor considered winter kill, poor seedbed preparation, and low soil fertility as the primary reasons for the failure of alfalfa. The winter hardiness problem was solved by the introduction of Grimm alfalfa in New Hampshire in 1915 (Prince, 1920).

Following a series of fertilizer experiments by the New Hampshire Agricultural Experimental Station, the use of alfalfa increased significantly in the state (Prince et al., 1942). In 1920, there were 3,000 acres of alfalfa in New Hampshire; by 1950, the acreage had increased to 12,000 (Table 1). At the present time, alfalfa accounts for 26 percent of the hay land in New Hampshire and yields average 5.8 t/ha (Crop Production, USDA Annual Summary, 1978).

Table 1. The acres of total hay and alfalfa in the United States, the North Atlantic States,[1] and New Hampshire from 1920 to 1978 at ten-year intervals. Yield data for alfalfa is also presented.

	Total hay (Acres x 1000)			Alfalfa (Acres x 1000)			Alfalfa (Yield, t/ha)		
Year	US	NA	NH	US	NA	NH	US	NA	NH
1920	56770	9805	372	9015	477	3	2.27	1.95	1.96
1930	55400	9916	378	10388	502	3	2.10	1.96	1.96
1940	59097	9258	356	13903	756	4	2.16	1.94	1.74
1950	65150	7995	272	19901	1556	12	2.14	2.01	1.75
1960	67313	6908	183	27580	1600	17	2.43	2.20	2.06
1970	62492	5968	124	27119	2206	21	2.93	2.64	2.38
1978[3]	60311	4926	72	26651	2000	19	2.91	2.69	2.55

[1]North Atlantic States: Maine, New Hampshire, Massachusetts, Vermont, New York, Connecticut, Rhode Island, New Jersey and Pennsylvania.

[2]Data taken from the Agricultural Statistics, USDA 1940 - 1979.

[3]Data for 1978 taken from Crop Production, 1978 Annual Report, USDA.

Soil Acidity

The soils of New Hampshire are acidic spodsols, highly weathered with illite as the major clay mineral (Gamble, 1954). The acidity is in part a function of the rain water leaching away the exchangeable bases replacing them with H and Al ions. Jenny and Leonard (1934) observed a correlation between the amount of rainfall and soil acidity. The authors indicated that the soil H ion content increased with rainfall. The source of H ions is the hydrolysis of water and the organic and inorganic acids produced by microbial action (Alexander, 1976). The Al comes from the weathering of the parent material by microbes (Alexander, 1976) and the hydrolysis with inorganic acids (Jackson, 1963).

Aluminum and Soil pH

Acidity measurements by pH are actually an expression of the H-ion content or activity in solution. When applied to a system involving two phases (soil and water) the meaning of pH is modified. The soil pH can be considered as a measure of H^+ activity when the liquid and solid phases are at equilibrium. The value obtained is the result of the amount of H ions dissociated from the soil complex and the extent of Al hydrolysis. Soil pH is not in any way a measure of Al but an expression of H ions regardless of their source.

The value of Al in soil acidity was not seriously considered until the introduction of saturated resin columns (Coleman and Thomas, 1967) even though some researchers had consistently shown the presence of Al in salt extracts from soil (Schofield, 1949). It was generally agreed that the Al was an artifact of the extraction method in which exchangeable H^+ combined with the chloride salts producing HCl which solubilized the Al from the parent material (Black, 1968). Davis et al. (1962) passed bentonite clay through a H^+-saturated resin column. The authors measured titratable H+ with NaOH and exchangeable Al with $BaCl_2$ immediately and after 1, 2, 4, and 12 hours after heating at 95 ^oC. If HCl was responsible for the Al presence, then the levels of the element should not have been increased by the heating. The results showed that the heating increased the levels of exchangeable Al over time, and the titration curves were markedly different. Lin and Coleman (1960) leached bentonite clay with $AlCl_3$, washed out the excess with water and extracted the clay with equal portions of $BaCl_2$, KCl and $CaCl_2$. The three extracts removed equal amounts of Al and the milliequivalents (Meq) of Al were identical to the Meq of Ca if the clay was initially leached with $CaCl_2$. The experimental data clearly demonstrated that the Al was not an artifact and that the element was exchangeable.

Exchangeable and Non-exchangeable Aluminum

Aluminum exists in the soil in exchangeable and non-exchangeable forms (Jackson, 1963). Exchangeable Al is postulated to exist primarily as $Al(H_2O)_6^{+3}$ and Al hydroxyl polymers on clay and organic matter are thought to be non-exchangeable Al (McLean et al., 1965). The exchangeable Al is part of a coordination complex in which the element is bonded to the oxygen molecule of water. This hydrated compound is both an exchangeable cation and an acid due to its ability to donate protons:

$$Al(H_2O)_6^{+3} + H_2O \rightarrow (Al(H_2O)_6OH)^{+2} + H^+$$

The further addition of hydroxyls produces complex Al ions that absorb on clay and organic matter and do not take part in cation exchange. Weissmuller et al. (1967) suggest that these complexes are composed of a series of hexagonal rings of Al ions in which only a portion of the ring is hydroxyls, so that the ring structure is charged.

Non-exchangeable Al is considered to play in important role in the soil cation exchange capacity (CEC). This is clearly shown in the influence of extraction procedure on the soil CEC. Bhumba and McLean (1965) demonstrated that unbuffered KCl-CEC was increased by liming and that organic matter in the soil was responsible for the pH dependency. Extraction with NH_4OAC at pH 7.0 was not influenced by pH or organic matter. Helling et al. (1964) measured the KCl-CEC of 60 Wisconsin soils and determined the contribution of organic matter and clay to the increase in CEC after liming. According to the authors, between pH 5.0 and 7.0, the majority of the increase in CEC was due to organic matter. McLean et al. (1965) indicated that the effect of organic matter on CEC is solely dominated by Al. This Al is tightly held to the organic matter and is not extractable by KCl.

Aluminum and Phosphorus Fixation

Several authors have discussed the relationship of Fe and Al to P fixation in soils (Hsu, 1964; Low and Black, 1950; Jackson, 1958). According to Vijayachandran and Harter (1975), the role of Fe in the fixation of P is considered less important than Al in temperate soils. In eleven soil samples collected from the United States and Puerto Rico, the authors found significant correlations between exchangeable Al and P fixation but with Fe, no significant correlations to P were found. Similar results were found for Florida soils (Yuan and Breland, 1969) and for New Brunswick soils (Saini and MacLean, 1965).

There is a general debate in the literature as to the process of P fixation: precipitation and/or adsorption. It is acknowledged that P fixation proceeds in two steps: a fast phase that reaches completion in minutes or hours and a slower phase that requires months (Hsu, 1964 and 1965; Hsu and Rennie, 1962). Some researchers suggests that the immediate reaction is the adsorption of P by hydroxyl Al polymers and the slower is the eventual precipitation of $Al(OH)_2H_2PO_4$ (variscite). Hsu (1965) states that the differences in reaction rate are determined by the readiness of Al to react. Experiments by Haseman et al. (1950) showed that the fixation of P was not dependent on the initial P concentration; this is typical of surface adsorption phenomena. Hsu contends that all P fixation is adsorption by Al polymers and that variscite never forms in the soil. Lindsay et al. (1959) state that the immediate reaction products of applied P in an acid soil are too soluble to be variscite but upon ageing these products are transformed to variscite. Veith and Sposito (1977) gave evidence for the formation of X-ray analogs of variscite under controlled conditions.

Soil Aluminum and Legume Growth

Acid soils are generally associated with low levels of Ca and P and toxic levels of Al and Mn (Andrews, 1977). Extensive studies have examined the influence of these factors on the growth of many non-leguminous horticultural and agricultural crops: corn (Fox, 1979; Ragland and Coleman, 1959): wheat (Lafever et al., 1977; Kerridge and Kronstad, 1968; Mugwira et al., 1976); cotton (Adams and Wear, 1959; Foy and Brown, 1964); barley (Rees and Sidak, 1961; Reid et al., 1969); rice (Howden and Cadavel, 1976); sugar cane (Juang, 1973); sorghum (Reeve and Sumner, 1970a); carrots (Gupta and Chipman, 1976; Gupta et al., 1970); citrus (Liebig et al., 1942; and Guest and Chapman, 1944); peach trees (Horton and Kilpatrick, 1976; and Jones and Jones, 1974); sugar beets (Kesar et al., 1977) and tomatoes (Kirsch et al., 1960; Foy et al., 1973). The general opinion is that Mn and/or Al induce stunting, depress root elongation, inhibit lateral and root hair development and reduce the levels of Ca and P in plant tissue.

For legumes, studies on soil acidity must consider the host plant, the specific Rhizobium and the symbolic relationship leading to nodulation and nitrogen fixation. Several authors have shown that applied N reduces pH effects and to a lesser extent Al toxicity for legumes (Munns, 1965a, 1965b; Loneragan and Dowling, 1958; Andrews and Johnson, 1976). The implications are that the primary effects of soil acidity for legumes are on nodulation and nitrogen fixation.

The interaction of Ca, P, H, Al, and Mn on yield and root development are generally known. Also the influence of Ca, P, H, and to some extent, Mn on nodulation is known; but the effects of Al on nodulation is still unclear. The difficulty lies in the problem of separating Al-P interactions from Al toxicity in solution and in the soil. In a series of hydroponic studies, Munns (1965a, 1965b) showed that alfalfa and subterranean clover had different reactions to pH. Alfalfa would grow at pH 4.0 and 5.0 if supplied with N and if Al was not present. Added Al decreased the growth of alfalfa supplied with N and totally inhibited the growth of the plants dependent on nitrogen fixation. The clover was not influenced by the pH either with or without N, and the Al was harmful only at the highest concentrations. In these solutions, Munns could not separate Al effects from an Al induced P deficiency. The author had shown the presence of Al-P colloidal compounds in solution that were colorless and difficult to detect. Only at pH's below 5.0 could the adverse influence of Al be separated from the P deficiency and Munns showed that alfalfa plants did not show any reduction in levels of P at pH 4.5. There is some indication, however, that the concentration of P in plant tissue may not be a reliable method to determine P deficiency due to Al-P interactions in the plant. Clarkson (1965, 1967) showed the presence of Al-P precipitates in onion root cell walls. McCormick and Borden (1974) used transmission electron microscopy with an electron dense strain to show the presence of Al-P precipitates in the intracellular spaces of barley roots.

In nodulation of alfalfa, Ca countered the effects of pH between 4.8 and 5.6. Below pH 4.8, no reasonable level of Ca could stimulate nodulation and at Ca levels less than 02. mM, nodulation would not occur at any pH (Munns, 1965c). Loneragan and Dowling (1958) found similar results with subterranean clover, but the critical levels of pH and Ca were different. Nodulation would not occur below pH 4.0 and 0.01 mM Ca. Munns (1970) demonstrated that alfalfa released a pectinase during the formation of the infection thread by the Rhizobium and postulated that the enzyme had a Ca requirement. The acid sensitive stages of nodulation for several legumes have been established. In Pisum (Lie, 1969), Trifolium (Lowther and Loneragan, 1970) and in Medicago (Munns, 1969), this period is within one to three days after inoculation, which corresponds to root hair curling and the initiation of the infection threads.

Loneragan and Dowling (1958) contend that the level of Ca required for nodulation is higher than that required for growth by the host plant or the Rhizobium. Norris (1959) examined the Ca and pH requirements of 48 species of Rhizobium and found that pH modified growth significantly but there was no detectable Ca requirement. Recently Keyser and Munns (1979a) studied the influence of Ca on the growth of 23 cowpea and 10 soybean Rhizobium strains. The authors found that low Ca levels reduced the growth of only one cowpea and three soybean Rhizobium strains.

The influence of Mn on nodulation is unclear. A study by Andrews (1977) on tropical legumes showed that 1600 uM of Mn reduced nodulation only by 7 percent in comparison to the controls. Vose and Randall (1963), however, found that Mn levels of 200 uM severely reduced the nodule number in white clover. In agar slants, 400 uM of Mn delayed nodulation in subterranean clover (Andrews and Hegarty, 1969). Differences in response between legumes may be due to variation between plants or a variation in growth habits of temperate and tropical legumes.

The influence of Al on nodulation and nitrogen fixation has not been clarified. Jensen (1948) indicated that once alfalfa was nodulated, the plant could fix nitrogen at pH 4.5; but these experiments were carried out without Al. An approach to clarifying the role of Al in nodulation would be to nodulate the plants in sand and then transfer them to water culture at desired Al levels, keeping the solution at pH 4.5 and 5.0.

There is very little evidence for a higher requirement for P in nodulation than in regular plant growth. But Gibson (1977) has shown nodulation to be an expensive process and Moustafa and Boland (1971) used foliar sprayed ^{32}P to demonstrate that P is translocated to the nodules in large amounts. The majority of the recovered ^{32}P was in AMP, ADP, and ATP. Jones et al. (1977) found that nodule number and weight in soybeans increased with added P. deMooy and Pesek (1966) state that the benefit of P in nodulation may be due to other soil factors that interact with P. It would be very difficult to separate the influence of P on dry matter accumulation from the increases in nodule number.

Several papers have reported increased nodulation in soil with reduced Al saturation (Moschler et al., 1960; Rice, 1975; Rice et al., 1977). The difficulty with defining Al toxicity in soil is in separating not only Al-P interactions but Al-Ca interactions as well. Zakaria et al. (1977) showed that soybean nodule number and weight increased with decreasing Al saturation, but levels of Ca and P also increased in the soil. Sartain and Kamprath (1977) found that soybean nodule number improved with reduced Al saturation, but the concentration of Al in the nodules was not lessened. At all levels of Al saturation, the levels of Al in the nodules remained the same. The authors offered no explanation for this situation.

The effects of Al and P on Rhizobium growth were recently examined in culture by Keyser and Munns (1979a, 1979b). The researchers grew cowpea and soybean Rhizobium strains in the presence of Al at pH 4.5. Tolerance to low pH did not necessarily include tolerance to Al; the Al increased the lag time in the bacterial growth and lessened the total number of bacteria over the growth period compared to the controls. The Al limited growth to a greater extent than low P or acidic pH. Possibly the major effect of Al in acidic soils is in the inhibition of Rhizobium growth. Rhizobium meliloti is known to be the most sensitive species to acidic pH, a characteristic that it shares with the host plant, alfalfa. Vincent (1977) noted that the nature of the acidity tolerance of the host plant and its specific Rhizobium are usually correlated. This correlation is not entirely a function of H ion activity since many legumes will grow at lower pH in solution than their respective Rhizobium. The sensitivity of R. meliloti to Al is not known, but the information would be helpful in clarifying the role of Al in acid toxicity of alfalfa.

Lime Chemistry and Practices

Lime was associated with the growing of alfalfa long before the theories on soil acidity were introduced. Early farmers in New England were aware that alfalfa was a lime-loving plant. The early success of this legume in New York is thought to be due to plantings in soils high in Ca. Taylor (1910) points to low soil fertility and acidic pH as limiting factors for the failure of alfalfa in New Hampshire. Liming soil increasesexchangeable bases, reduces Al and Mn levels, decreases anion adsorption releasing them into solution, and improves conditions for microbial action. The effects of these changes varies between legumes depending on their tolerance to soil acidity.

Lime materials and practices are dependent on cost, soil pH, and the crop. The most commonly used limes in New England are in order: dolomitic ($CaCO_3$, $MgCO_3$), calcitic ($CaCO_3$), and hydrated ($Ca(OH)_2$). These materials differ in neutralizing power, which is a function of solubility and particle size. Calcite is assigned a value of 100 for neutralizing power, dolomite a value of 95 to 108, and hydrated a value of 134. All lime should be fine enough that 90 percent passes a 10-mesh screen, 50 percent a 50-mesh screen, and 25 percent a 100-mesh screen (Jones, 1979).

Lime can be applied by broadcasting, pelleting, and banding. Lime pelleting of seeds was first used in Maine in 1941 with alfalfa in acidic soils (Brockwell, 1963). Since then, the practice has spread to many other places and is now used extensively in Australia and New Zealand (Brockwell, 1977). The advantages of pelleting are that the

Rhizobium are in an environment high in Ca that is protected from microbial predation and competition. Pelleting is used frequently with seeding of clovers into rangelands in Australia (Munns, 1977). This method has been found to be less beneficial to alfalfa because toxic levels of Al and Mn are not reduced. Norris (1965) considers the use of lime pelleting harmful to acid tolerant legumes, especially the tropical group which tend to have a lower Ca requirement. These legumes are usually nodulated by alkaline-producing Rhizobium that do not thrive at higher pH levels such as lime pelleting would create. In contrast to the above negative response, Cordero and Blair (1978) working with tropical legumes found that lime pelleting the seed caused significant increases in nodulation in the clovers tested. The benefit of pelleting was reduced acidity and higher levels of Ca in the immediate area of the seed. Banded lime has been used to some extent with alfalfa in acidic soils. The principal is similar to pelleting; i.e., induce a high level of Ca in the area of the seed for nodulation. Brown (1950) found that banded lime was not beneficial in establishing alfalfa but was quite effective in stand maintenance and yield. There are no reports of banded lime being used with clover.

Generally lime is applied by broadcasting in split applications with plowing and disking to attempt an even mixture in the soil. Several studies have shown that pH adjustment proceeds much faster in greenhouse studies than in the field because lime can be more evenly mixed under greenhouse conditions (Jones, 1979; McLean, 1976). Even with poor mixing, pH chnages with broadcast lime are usually rapid in temperate areas. Prince et al. (1942) limed a field, initial pH

5.3, with 2.3 and 4.6 metric tons per hectare (t/ha) of calcite in 1928. Within one year the pH at 2.3 and 4.6 t/ha was 5.7 and 6.0, respectively; and after two years, the pH at both lime rates exceeded 6.0.

Brown et al. (1956) applied 4.6, 9.2, and 13.8 t/ha calcite to a grass sod and measured the pH over a ten-year period. The authors found that the rate of lime had less effect on the pH than time. The soil pH changed as much by the low lime rate as the high rate in the study period. A similar study was done by Longnecker and Sprague (1940) with a series of soils ranging from a loamy sand to a clay loam. The authors found that lime penetration was dependent on soil type and lime. The results from both these studies indicate that pH change in a grass sod is a long-term process. Under this situation, lime pelleting or banding might be beneficial especially for alfalfa.

There is considerable debate in the literature as to the final pH to which a soil should be limed. The degree to which P fixation is relieved by liming is at the center of the issue. Reeve and Sumner (1970a) demonstrated with several Florida soils that the levels of exchangeable Al were practically eliminated by liming but there was no increase in extractable P. Munns (1965b) found that lime reduced Al saturation but had no influence on the release of P in the soil. Reeve and Sumner (1970b) stated that since liming did not influence P, only the reduction of Al levels should be the goal. Kamprath (1970) stated that the reduction of Al saturation should be the aim of liming. Kamprath found in a series of experiments that maximal crop yield could be achieved at a pH lower than normally limed for once Al saturation had been reduced. McLean (1976) suggests that acid soils should be limed to pH 6.5 to increase the availability of micronutrients, improve nitrogen mineralization and reduce Al saturation. McLean believes that liming only to eliminate Al might be beneficial in tropical soils but not in temperate soils.

Munns (1965b) demonstrated that the lime response in clover could be completely eliminated by massive applications of P. For alfalfa, increased levels of P had no influence until Al saturation was totally reduced. Coleman et al. (1959) demonstrated that in several soils from North Carolina Al was reduced to less than 10 percent saturation by pH 5.6. Cordero and Blair (1978) found that pelleting clover seed with lime and superphosphate was sufficient for good yields in acidic soils. The data from the literature indicate that the interaction of lime and P in improving growth of legumes in acidic soils is still open to question.

II. MATERIALS AND METHODS

Greenhouse Investigations

Influence of Lime on Soil Acidity and Legume Growth

Of the greenhouse experiments, three were conducted to clarify the influence of lime and P on the nodulation, nitrogen fixation, and yield of red clover and/or alfalfa.

Study 1: The Response of Alfalfa to Lime. Three soils were collected from sites that were not in current use. The soils and sites selected were: Agawam silt loam, Claremont, New Hampshire; Charlton loam, Lee, New Hampshire; and Paxton silt loam, Pittsfield, New Hampshire. The top 15 cm of the soil profile was collected from each location in August 1976, screened and sampled on site. In September 1976, dolomitic limestone at 0, 4.6, and 9.2 t/ha and 120 kg K_2O/ha were thoroughly blended with the above soils; and the resulting mixture was placed into 200 mm plastic pots. The potted soils were watered to allow the lime to solubilize until December 1976 when Saranac alfalfa was inoculated and seeded. The pots were placed on a greenhouse bench under cool white fluorescent lights with a 16-hour daylength. Ten days after emergence, the plants were thinned to five plants per pot. The experimental design was a random complete block with four replications.

The plants were harvested in March (tops) and in May (whole plants). In May, nodule number, weight, color, and nitrogen fixation were determined along with yield. Nitrogen fixation was measured via acetylene reduction using the procedure of Hardy et al. (1968). This technique is based on the fact that the nitrogenase enzyme will reduce acetylene (C_2H_2) to ethylene (C_2H_4):

$$N_2 \xrightarrow[N_2\text{ase}]{6\ e^-} NH_4^+$$

$$C_2H_2 \xrightarrow{2\ e^-} C_2H_4$$

The ratio of C_2H_2/N_2 is considered to be 3:1, but research has shown that the ratio varies between species, with the stage of plant growth and with environmental conditions (Hardy et al., 1968).

The acetylene used in these experiments was generated by the reduction of calcium carbide with water:

$$CaC_2 \quad + \quad 2H_2O \quad \rightarrow \quad Ca(OH)_2 \quad + \quad C_2H2$$

The five plants were carefully removed from the soil and the roots were separated from the tops. The nodulated roots were gently washed with water to remove soil and placed in Mason jars (250 ml) modified with 5.0 cm lengths of copper tubing through the center of the lids. The outer end of the tubing was sealed with a serum cap which allowed gas to be injected and sampled. From the jars containing the roots, 25 ml of air was replaced with an equal volume of generated C_2H_2 with a disposable syringe. The jars were incubated at 28^o for 60 minutes in a water bath. After incubation, three 1 ml gas samples were withdrawn and analyzed for C_2H_4 against 1 ppm standards with a

Perkin-Elmer Gas Chromatograph Model 990 on an alumina column at 100 °C.

After the gas samples were taken, the nodules were removed from the roots, counted, weighed, and the percent of the total nodules that were pink was determined. The pink color is considered as an indication of the presence of legahemoglobin and active nitrogen fixation (Date, 1970).

Soil samples taken prior to liming and after final harvest were analyzed for pH, Ca, Mg, K, P, and Al. Soil pH was determined in a 1:1 water-soil slurry with an Orion Digital pH Meter. Exchangeable Ca, Mg, and K were extracted with 1 N NH_4OAC and determined by atomic absorption or flame emission. Lanthanium chloride was added for the analysis of Ca and Mg at a final concentration of 0.1 percent to prevent the formation of phosphate compounds with these elements (Skoog and West, 1974). Exchangeable Al was extracted with 1 N KCl and measured by atomic absorption using nitrous oxide as the oxidant. Available P was extracted with a Bray #1 solution (Bray and Kurtz, 1945) and measured according to the method of Olsen and Dean (1969).

The data were analyzed with Duncan's New Multiple Range Test and multiple regression analysis.

Study 2: The Response of Alfalfa and Red Clover to Lime Pelleting. Paxton silt loam soil which had received 0, 4.6, and 9.2 t/ha dolomitic limestone was used in this experiment. The soil was placed in 200 mm plastic pots and fertilized with 600 kg 0-20-20/ha. Seedings were made of both pelleted and non-pelleted Saranac alfalfa and Pennscott red clover in September 1977. The pelleting procedure consisted of three basic steps: 1) 20 grams of gum arabic were dissolved in boiling water and cooled. 2) approximately one pound of legume seed and one package of appropriate Rhizobium were mixed in the cooled arabic. 3) the Rhizobium coated seeds were rolled in dolomitic limestone. The first preparation resulted in severe clumping and the procedure was repeated allowing the free moisture to be absorbed before rolling the seeds in the limestone; this procedural change prevented the clumping.

The plants were kept on a greenhouse bench under cool white fluorscent light with a 16-hour daylength and watered as required. The experimental design was a random complete block with four replications. The study was harvested in December 1977 and analyzed for yield, nodule number, weight, and nitrogen fixation with the procedure outlined in Study 1.

Study 3: The Response of Alfalfa to Lime and Superphosphate. Five rates of dolomitic limestone (0, 2.3, 4.6, 9.2, and 18.2 t/ha), hydrated limestone (0, 1.2, 2.3, 4.6, and 9.2 t/ha) and triple superphosphate (0, 0.16, 0.32, 0.62, and 1.30 t/ha) were thoroughly mixed with a Paxton silt loam soil (pH 5.6) on December 27, 1978. The soil was placed into plastic-lined 200 mm plastic pots, seeded with inoculated Iroquois alfalfa and placed on a greenhouse bench. Soil samples were taken for Al on January 3 and February 1, 1979; for P on January 3 and February 28; and for pH on December 29, 1978, January 3, February 1, and March 4, 1979, using the procedure discussed in Study 1. The experimental design was a random complete block with five replications.

In May, the plants were removed from the pots and the roots were separated from the tops. Nodules were counted and weighed. The plant tops and roots were oven dried at 70 °C for 48 hours, weighed, ground in a Wiley Mill to pass a 40-mesh screen, ashed at 450 °C for three hours, and analyzed for Ca, Mg, Al, Zn, and Fe with atomic absorption. Lanthanium chloride was added for Ca and Mg analysis. Plant P was measured with molybdate yellow according to Chapman and Pratt (1961). In order to have sufficient material for tissue analysis, it was necessary to combine the five replications.

The data for yield and nodulation were treated with analysis of variance and Duncan's New Multiple Range Test.

The Effects of Aluminum on Aeroponically Grown Alfalfa and Red Clover

Hydroponics has been used extensively in the study of nutrient effects and interactions on legume growth (Lie, 1969; Loneragan and Dowling, 1958; Munns, 1965a, 1965b, 1965c, 1968, 1969, 1970). The water environment of the hydroponic culture is usually not aerobic enough to allow nitrogen fixation since the water nodule surface interaction prevents adequate gas diffusion (Sprent, 1976).

Zobel *et al*. (1976) constructed a system in which the plant roots were misted with nutrient solution providing a highly aerobic environment for root hair development, nodulation, and nitrogen fixation. This aeroponic system was employed to study the influence of Al on nodulation and nitrogen fixation of alfalfa and red clover.

The mist system consisted of boxes (61 x 122 x 46 cm) constructed from 0.64 cm plywood and lined with a double layer of 4 mil polyethylene sheeting. A plastic spinner (Northern Electric Co., Waynesboro, Mississippi) attached to a small moter (1/40 hp, 115 v and 3905 rpm) via a stainless steel shaft (1.3 cm in diameter, 30 cm in length) was the misting mechanism. The spinner dipped into the nutrient solution, atomized it, and misted the plant roots. Plastic grating (1/4" squares) used in light fixtures was fitted over the top of the boxes and covered with one layer of black 4 mil plastic sheeting and one layer of 4 mil clear plastic sheeting.

Saranac alfalfa and Pennscott red clover were inoculated and seeded into sand. Seven days after emergence, nodulated legumes were transferred to the aeroponic boxes. Holes were cut in the plastic sheeting such that plants were 5 cm apart; plants were held in place with non-absorbent cotton. Each box contained 40 liters of nutrient solution adjusted to pH 5.0 (Table 2). Root debris caused the spinner to clog, and a stainless steel mesh screen cage was constructed to surround the spinner, thus correcting the problem. Forty-eight hours after the plants were introduced to the boxes, the nutrient solution was changed and four conditions were established: 0 ppm Al, 1 ppm Al, 2 ppm Al, and 2 ppm Al + N (Table 2). Nitrogen was added as a control to determine how its addition influenced Al toxicity. The nutrient solution was changed weekly. Four boxes were built and a latin square (4 x 4) design was used, with replications over time.

Each box contained 40 plants, 20 of each legume, randomly distributed. Every week, four plants per legume were removed from each box and measurements were taken for yield, nodule number, weight, and nitrogen fixation. The plant tops were dried at 70 ^{o}C for 48 hours and weighed. The nodulated roots were placed into 200 ml vials (two plants/vial), sealed with a serum cap and C_2H_2 generated from calcium carbide was used to replace 20 ml of air in the vials. The vials were

Table 2. The nutrient solution used for the aeroponic culture of alfalfa and red clover.

Stock	+N		-N
		ml/liter	
1 M $Ca(NO_3)_2 \cdot 4H_2O$	5		-
1 M KNO_3	5		-
1 M $MgSO_4 \cdot 7H_2O$	2		2
1 M KH_2PO_4	1		1
Sequestrene Fe-330	1		1
1 M $CaCl_2 \cdot 2H_2O$	-		5
1 M KCl	-		5
Micronutrients*	1		1
1 M $Al_2(SO_4)_3 \cdot 18H_2O$		0, 0.02, 0.04	

* Micronutrient solution contained 2.86 g H_3BO_3, 1.81 g $MnCl_2 \cdot 4H_2O$, 0.11 g $ZnCl_2$, 0.05 g $CuCl_2 \cdot 2H_2O$, 0.024 g $NaMoO_4 \cdot 2\ H_2O$ and 0.005 g $CoCl_2 \cdot 6H_2O$ per liter.

incubated for 60 minutes in a water bath at 28 ^{o}C following which three 1 ml gas samples were taken from each vial with 1 ml tuberculin syringes. The samples were analyzed for C_2H_2 against 1 ppm standards as previously described. Nodule number and weight were recorded for each treatment and the data within each replication were combined.

In four weeks, at the completion of each replication, the boxes were thoroughly cleaned and the plastic linings replaced. For each replication, new legume seedlings were used so that plant age would be consistent throughout the experiment. The study was conducted from January to May 1978.

The Effect of Fertilizer Nitrogen on Nitrogen Fixation and Nitrate Reduction in Alfalfa

Munns (1965c) demonstrated that the addition of N eliminated the lime response in alfalfa at low levels of exchangeable Al. This experiment was conducted to determine the effects of applied N on nitrogen fixation, nodule number and nitrate reduction of alfalfa and red clover in a moderately acid soil.

A Charlton loam soil whose chemical characteristics are shown in Table 3 was mixed with an equal part sand to give a soil low in N. The soil was fertilized with 600 kg 0-20-20/ha and 2.2 kg B/ha from Solubor. Nitrogen was added at 60 kg N/ha as NH_4NO_3 when used. All fertilizers were mixed evenly with the soil before potting. Saranac alfalfa and Pennscott red clover were inoculated and seeded into the various soil treatments in 200 mm plastic pots. The pots were kept on a greenhouse bench under cool white fluorescent lights at 16-hour daylength. The experimental design was a random complete block with three replications.

Table 3. Chemical analysis of the Charlton loam soil used in the Greenhouse Experiment

pH	Ca	Mg	K	Al	P	NO_3[1]
	Meq/100 g				ppm	
5.6	3.97	1.34	0.97	0.04	28	low

[1]Analysis done by Analytical Services, Plant Science Department using the Morgan Soil Testing System.

Every week each treatment with replications was harvested and the plants measured for yield, nodule number, weight, and nitrogen fixation as previously described. Nitrate reductase activity in alfalfa and red clover was measured with the procedure from Nicholas et al. (1970). In this method, the leaves are removed from the legumes and a cork borer is used to punch out approximately 200 mg of tissue. The tissue is incubated in 10 ml of 50 mM KNO_3, 100 mM K phosphate buffer (pH 7.5) and 1 percent propanol (v/v). The tubes containing the leaf tissue and the media are placed in a vacuum desiccator and infiltrated twice (two minutes each) and then placed in the dark in a shaking water bath at constant temperature (25 ^{o}C) for 60 minutes. The NO_2 that diffuses into the media was analyzed according to Harper and Hageman (1972). Preliminary experiments indicated that two modifications of this procedure had to be made to obtain satisfactory results for alfalfa and red clover. In order to get sufficient infiltration of the media into the leaf disks, stainless steel mesh squares were used to keep the tissue below the media surface and the infiltration time under vacuum was increased to five minutes. With these changes, the procedure worked quite well.

The data were treated with analysis of variance and Duncan's New Multiple Range Test.

The Influence of Soil Amendments and Rhizobium Strain on the Nodulation of Alfalfa and Red Clover

These two experiments were conducted to study the influence of *Rhizobium meliloti* strain on the growth of alfalfa and to evaluate the influence of lime and phosphorus on the effectiveness of *Rhizobium* to nodulate alfalfa and red clover.

Study 1: The Interaction of Rhizobium meliloti Strain, Alfalfa Variety and Soil pH. A Charlton loam soil was autoclaved to remove native *Rhizobium* populations, limed with dolomitic limestone at 0, 6.9, and 13.8 t/ha and fertilized with 600 kg 0-20-20/ha. Three varieties of alfalfa were used in the experiment: Saranac, a commercial variety; NK7-5 received from Northrup King Co., Minneapolis, Minnesota; and Arc A1-14 received from Dr. James H. Elgin, Jr., Plant Genetics and Germplasm Institute, Beltsville Research Center, Beltsville, Maryland. The seeds were surface sterilized by immersion in 1 percent $HgCl_2$ for 20 minutes, followed by four ten-minute washes in sterile distilled water. The seeds were planted directly or inoculated with one of seven strains of *Rhizobium meliloti*. The strains were received from

Nitragin Co., Milwaukee, Wisconsin, in 1978 and were designated 102F51, 102F52, 102F66, 102F70, 102F73, 102F77, and 102F82. The strains were received on agar slants, transferred to yeast mannitol broth, and stored at 4 °C until used. The sterilized seeds were inoculated by soaking for 20 to 30 minutes in flasks containing a single R. meliloti strain in yeast broth media. The control was an application of 120 kg N/ha applied as NH_4NO_3 at seeding. The plants were seeded into 102 mm plastic pots, thinned to five plants per pot after emergence, and kept under cool white fluorescent lights set at 16-hour daylength on a greenhouse bench. The experimental design was a random complete block with five replications.

After eight weeks, the plants were harvested for yield, nitrogen fixation, and nodule number as in Greenhouse Experiment Study 1 concerning the influence of lime on soil acidity and legume growth.

Study 2: The Influence of Applied Lime and Phosphorus and the Presence of the Host Plant on the Effectiveness of Rhizobium in the Nodulation of Alfalfa and Red Clover. The soil was collected from two field experiments discussed later in Materials and Methods. At the Harmony Farm site, soil was collected from the three seeding dates at 4.6 and 13.8 t/ha dolomitic limestone. At the Demerit Farm site, soil was collected from the unlimed plots, 2.3 t/ha hydrated lime and 13.8 t/ha dolomitic lime plots which received three rates of broadcast P: 0 kg P_2O_5/ha; 220 kg P_2O_5/ha; and 440 kg P_2O_5/ha.

At both sites, the top 10 cm of the soil profile was collected, transported in plastic bags, screened in the greenhouse, and placed into 102 mm plastic pots. Iroquois alfalfa and Pennscott red clover seed were inoculated and placed into their respective soil; uninoculated checks were also used. After emergence, the plants were thinned to five per pot and watered as required. The experimental design was a random complete block with five replications.

The influence of applied lime and P was associated by evaluating the nodules of three plants per pot for color, position, number and size using a scoring system devised by Rice et al. (1977). In the system used by Rice et al., alfalfa and red clover were evaluated using the same ranges for the four factors. Field and greenhouse observations have shown that red clover generally had more nodules per plant than alfalfa so in this experiment, the ranges for nodule number were different for the two legumes (Table 4).

Scanning electron microscopy was used to examine the nodules taken for alfalfa and red clover grown in the soil collected from the Harmony Farm. Nodules were selected from both lime rates at the May 1978 and 1979 seeding dates and were representative of the nodulation pattern of the plant. The procedure used to prepare nodules for scanning electron microscopy is as follows:

1. 50 minutes in 3.0 percent glutaraldehyde in 0.1 M phosphate buffer, pH 7.5.
2. 30 minutes in 1 percent OsO_4 in 0.1 M phosphate buffer, pH 7.5.
3. 15 minutes in 0.1 M phosphate, pH 7.5.
4. repeat procedure 3.
5. alcohol dehydration series - 15 minutes in each of the following: 70 percent, 75 percent, 80 percent, 85 percent, 90 percent, 95 percent, 99 percent, and 100 percent (twice).
6. critical point dry - 15 to 20 minutes to exhaust.

Table 4. The scoring system used to evaluate nodules.

Factor	Code	Alfalfa	Red Clover
Color	4	80 - 100% pink	same
	3	60 - 79 "	
	2	40 - 59 "	
	1	20 - 39 "	
	0	0 - 19 "	
Number	5	> 20/plant	> 40/plant
	4	15 - 20 "	20 - 40 "
	3	10 - 14 "	10 - 19 "
	2	5 - 9 "	5 - 9 "
	1	1 - 4 "	1 - 4 "
	0	0 - 1 "	0 - 1 "
Position	4	80 - 100 crown	same
	3	60 - 79 "	
	2	40 - 59 "	
	1	20 - 39 "	
	0	0 - 19 "	
Size	3	> 4 mm	same
	2	2 - 4 "	
	1	1 - 2 "	
	0	0 - 1 "	

The nodules were mounted on aluminum stubs with double-sided tape and were coated with gold-palladium (60/40) on a sputter coater. The nodules were scanned with an AMB-1000 Scanning Electron Microscope.

The Influence of Aluminum on the Mobility of Phosphorus-32 in Red Clover and Alfalfa

Since Al hinders P availability in acidic soils, tolerance to low pH is known to include the ability to utilize P in the presence of Al (Foy et al., 1978). This experiment was conducted to evaluate the influence of Al on the mobility of P in alfalfa and red clover in the presence of Al. Electron dispersion analysis via X-ray (EDAX) and ^{32}P were used to evaluate the movement of P in the legumes.

Saranac alfalfa and Pennscott red clover were inoculated and seeded into acid washed gravel in January 1979. Two weeks after emergence, the plants were transferred to a hydroponic system consisting of 500 ml Mason jars wrapped in aluminum foil and placed on glycine trays in the greenhouse under cool white fluorescent lights at 16-hour daylength. Lighting grates were laid over the jars, and the plants were held in place by non-absorbent cotton. Each jar was aerated and filled with 490 ml of the nutrient solution described in Table 5.

After five days in this nutrient solution, Al was used as $Al_3(SO_4)_3 \cdot 16H_2O$ at 0, 100, and 200 uM Al with the solution pH adjusted to pH 5.0. After 24 hours, the solution was changed and nodules were collected for EDAX. The new solutions were spiked using $H_3{}^{32}PO_4$ at 10 uc ^{32}P/liter. After 48 hours, the plants were removed

Table 5. The nutrient solution used to grow alfalfa and red clover in hydroponic culture.

Stock	ml/liter
1 M $CaCl_2 \cdot 2H_2O$	5
1 M KCl	5
1 M $MgSO_4 \cdot 7H_2O$	2
1 M KH_2PO_4	1
Sequestrene Fe-330	1
Micronutrients*	1
1 M $Al_2(SO_4)_3 \cdot 16H_2O$	0, 0.04, 0.08

* The recipe for the micronutrients is shown in Table 2.

and the roots washed in cold P (1 M K_2HPO_4). The plants were then carefully wrapped in saran wrap and stored in the dark on ice.

Within 30 minutes, the wrapped plants were placed in film cassettes with X-ray film (Kodak, No-screen Film, NS-5T). The film was developed within 24 to 30 hours with the procedure described below:

1. Developer (Kodak D - 19) five to six minutes with continuous shaking.
2. Stop bath, 2 percent acetic acid, 30 seconds with continuous shaking.
3. Fixer (Kodak General Hardening Fixer)
   a. directly after stop bath, four minutes.
   b. second fix, ten minutes.
4. Wash with cold running water for 30 minutes.
5. Photoflo (Kodak 200 Photoflo) briefly.
6. Hang to dry.

The experimental design was a random complete block with three replications.

The nodules collected before the addition of ^{32}P were washed thoroughly in distilled water. The specimens were carefully sliced in half longitudinally with a stainless steel razor blade (one blade per nodule) and freeze dried with a Virtis Unitrap II at -50 ^{o}C and 30 microns vacuum for 72 hours. The dried nodules were mounted on carbon stubs with carbon paint, coated with carbon and scanned for Al and P against appropriate standards. The EDAX package on the AMR-1000 Scanning Electron Microscope is equipped with a ZAF correction which compares specimens to standards and corrects for atomic number (Z), absorption (A), and fluorescence (F).

Laboratory Investigation

The Effects of Aluminum and pH on the Growth of *Rhizobium meliloti* in Culture

The effects of acidity on *Rhizobium* is well documented (Vincent, 1977), but the interactions of Al with pH on the growth of these bacteria is not known. This experiment was conducted to examine the influence of Al and pH on the growth of *R. meliloti* in culture.

Three of the seven strains used in the previous greenhouse experiment (page 28), Study 1, were used in this experiment: 102F51, 102F70, and 102F82. The experimental media contained (per liter): sucrose, 10 g; Na-glutamate, 1.1 g; $MgSO_4 \cdot 7H_2O$, 250 mg; $CaCl_2 \cdot 2H_2O$, 150 mg; KNO_3, 100 mg; H_3BO_3, 30 mg; $ZnSO_4 \cdot 7H_2O$, 20 mg; $NaMoO_4 \cdot 2H_2O$, 0.25 mg; $CuSO_4 \cdot 5H_2O$, 0.04 mg; $CoCl_2 \cdot 6H_2O$, 0.025 mg; chelated Fe-330, 28 mg; thiamine-HCl, 10 mg; and biotin, 1.0 mg.

Munns (1965b) demonstrated that Al and P in solution formed gelatinous, colorless colloidal compounds if the pH and the concentration of the two elements was not carefully controlled. Munns indicated that Al levels could reach 100 uM if the P levels did not exceed 10 uM at pH 4.8. Preliminary research for this experiment had shown that Al levels of 40 uM could be used in solution at 100 uM P at 5.0, 5.5, and 6.0 without the formation of precipitates in a 24-hour period.

The basal media (minus Fe), P as KH_2PO_4 and Al as $AlCl_3$ were autoclaved separately for 30 minutes. The chelated Fe-330 was filter sterilized with a Millipore apparatus through a 0.30 micron filter and added after the media was autoclaved along with the Al and P. The solution pH was adjusted at this time with sterile 0.1 N NaOH.

Protein levels were used in this experiment as an indication of growth using the Lowry-Folin assay (Lowry et al., 1951). It was necessary to evaluate if Al had any effect on this assay since research has shown that several materials interfere in the formation of the color complex (Bradford, 1976; Gornall et al., 1949). A series of protein standards were prepared at 0, 40, and 400 uM Al and assayed with the Lowry-Folin procedure (Table 6). The data indicated that the level of Al used in this experiment did not inhibit the assay.

The prepared media was dispersed into sterilized 125 ml Erlenmeyer flasks and inoculated with the appropriate Rhizobium strain. The bacteria were taken from the agar slants and diluted with media to give an initial protein concentration of 2 to 3 ug/ml. The flasks were plugged with sterile cotton, covered with small beakers and placed in a shaking water bath at 25 ^{o}C for 24 hours. Every four hours samples were taken for protein analysis with sterile pipettes. Each strain was run separately, and the experimental design was a split plot with three replications.

Table 6. The influence of aluminum on the Lowry-Folin protein assay.

Protein levels mg/ml	Aluminun levels 0 uM	40 uM	400 uM
	% transmission		
20	93	90	95
40	80	80	83
80	74	72	72
120	62	63	65
160	53	54	58
200	44	45	38

Field Investigations

The Influence of Lime and Phosphorus on the Growth of Alfalfa and Red Clover in Acidic Soils

Two field experiments were established with minimum tillage to examine the influence of banded and broadcast limestone and P on the nodulation, composition, and yield of alfalfa and red clover in acidic soils.

Study 1: The Influence of Seeding Date, Banded and Broadcast Dolomitic Limestone on the Growth of Alfalfa and Red Clover. The experiment was established on Harmony Hill Farm, Pittsfield, New Hampshire. The experimental site was an old pasture being used for grazing; the soil type was a Paxton silt loam. The pH on the 0 to 10 cm depth of the soil profile was 5.0, Al saturation exceeded 20 percent, and the levels of available P averaged 7 ppm. The site was selected, staked, and sampled in August 1976.

The experimental design was a split block with randomized subplots and four replications. Broadcast lime was applied across the replications in one direction and seeding date at right angle to the lime. Banded lime was randomized within each treatment. Three seeding dates were used: May 1978, August 1978, and May 1979.

The experiment was originally designed to evaluate the influence of lime pelleting on the establishment of alfalfa and red clover into acidic soils. Broadcast dolomitic limestone was applied to the entire experiment on May 10, 1977, at 4.6, 9.2, and 13.8 t/ha. The plots were sprayed with Paraquat (1,1'-dimethyl-4,4'-bipyridinium ion, as dichloride salts) at 0.56 kg/ha on August 10, 1977. Lime pelleted

Iroquois alfalfa and Pennscott red clover were seeded using the Zip seeder (Midland Mfg., Mississippi) which is a single disk cutter producing six nine-inch rows. The plots were fertilized with 825 kg 0-20-20/ha on the day of seeding. The lime pelleting procedure was outlined previously on page 22.

In October 1977, the native grasses were growing back and suppressing the legumes. Pronamide (N-(1,1-dimethylpropynl) –3,5-dichlorobenzamide) was applied in November at the rate of 3.3 kg/ha to control the grasses.

In early May 1978, the alfalfa seeding was poor; the plants were stunted, showed signs of severe P deficiency and the upper leaves were yellowing. Even though the plants were well nodulated where pelleting was used, the plant growth was restricted by the acidic conditions and/or grass competition. The plots were sprayed with glyphosate (N-(phospho-nomethyl)glycine) at 2.24 kg/ha on May 22. On May 25, the plots were reseeded with alfalfa and red clover using the Zip seeder and dolomotic limestone was placed in the fertilizer box on the seeder and banded with seed at 990 kg/ha (Figure 1). In June, carbofuran (2,3 dihydro(2,2 dimethyl-7-benzofuranyl)methylcarbamate) was applied as a general protector against insects at 0.7 kg/ha. On August 8, glyphosate was applied at 2.24 kg/ha for the second seeding. Alfalfa and red clover were seeded along with banded dolomitic limestone (990 kg/ha) on August 11; all plots were broadcast fertilized with 825 kg 0-20-20/ha immediately before seeding.

The first harvest was taken on August 27 with the Carter harvester (Carter Mfg. Co., Lafayette, Indiana). The percent legume was estimated for each plot by four separate observers on the basis of

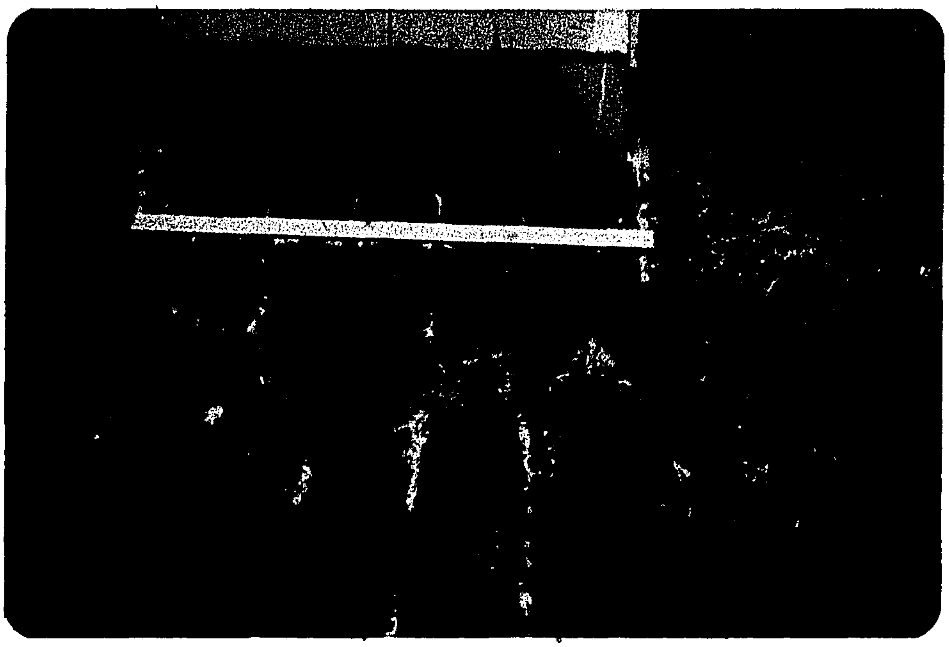


Figure 1. Applications of the banded dolomitic limestone adjacent to seed planted with the Zip seeder.

dry weight. Samples for dry matter determination and tissue analysis were taken after the harvest along the edges of the plots. The samples had to be hand collected because the harvester dusts the chopped legume with soil invalidating any tissue analysis for Al. All harvested plots were measured for length to accurately determine harvested area. All seeded plots were broadcast fertilized on August 30 with 550 kg 0-10-30-4/ha.

On May 5, 1979, glyphosate was applied at 2.24 kg/ha for the last seeding; and on May 8, alfalfa and red clover were sown with banded lime and the plots fertilized as previously described.

The first harvest of 1979 was taken with the Carter harvester on June 10 for the May and August 1978 seedings. Samples for dry matter determination were collected from the chopped legume, and the plots were measured for length. The second harvest (July 30) and the third harvest (October 23) were taken for all three seedings. Samples for dry matter and tissue analysis were hand collected from the second harvest.

In July 1979, whole plants (five/treatment) were dug up with a soil ball intact from all three seeding dates at 4.6 and 13.8 t/ha lime, placed in plastic bags, and transported to the greenhouse. The soil was carefully displaced with water from the roots, and the nodules were removed, counted, and weighed.

Soil samples were collected on six dates: May and September 1977, 1978, and 1979. Samples were taken at 0-2, 2-5, 5-10, and 10-20 cm into the soil profile and analyzed for pH, Ca, Mg, K, Al, and P. Soil pH was measured in a 1:1 water-soil slurry with an Orion Digital pH

Meter. The Ca, Mg, and K were extracted with 1 N NH_4OAC pH 7.0 and measured by atomic absorption or flame emission. Exchangeable Al was extracted with 1 N KCl and measured with atomic absorption. Available P was extracted using Bray #1 solution and determined according to the method of Olsen and Dean (1969).

Study 2: The Influence of Broadcast Limestone and Broadcast Phosphorus and Banded Phosphorus on the Growth of Alfalfa and Red Clover. The University of New Hampshire Demerit Livestock Farm, Lee, New Hampshire, was the site selected to study the influence of broadcast dolomitic and hydrated limestone in combination with broadcast superphosphate and banded monoammonium phosphate (MAP) on alfalfa and red clover in a moderately acid soil. The site was selected, staked, and sampled in August 1977. The soil was a Hinckley sandy loam with an initial pH of 5.6 at the 0-10 cm depth. The experimental design was a split block with P treatments applied across the replications in one direction and lime across the replications in the other direction. Because of a space limitation, the number of plots was restricted, and alfalfa and red clover were replicated four and three times, respectively.

On May 5, 1978, five lime treatments were established: 1) no lime; 2) 1.2 t/ha hydrated lime; 3) 2.3 t/ha hydrated lime; 4) 6.9 t/ha dolomitic lime; 5) 13.8 t/ha dolomitic lime. On August 14, 1978, glyphosate was applied to the plots at 2.24 kg/ha; and on August 17, Iroquois alfalfa and Pennscott red clover were seeded with the Zip seeder. Phosphorus was banded as a liquid using a hand sprayer attached to the rear of the seeder (Figure 2). The banded P was applied at 120 and 250 kg P_2O_5/ha. Broadcast P was applied the following day in such a way that six final P treatments resulted: 1) banded 240 kg P_2O_5/ha; 2) broadcast 440 kg P_2O_5/ha; 3) banded 120 and broadcast 220 kg P_2O_5/ha; 4) banded 120 and broadcast 440 pg P_2O_5/ha; 5) banded 240 and broadcast 220 kg P_2O_5/ha; 6) banded 240 and broadcast 440 kg P_2O_5/ha. Potassium was applied at a rate of 120 kg K_2O/ha to all plots.

The first harvest was taken on June 6, 1979, with the Carter harvester and samples for dry matter determination and tissue analysis were hand collected. One week after harvest, the plots were fertilized with 120 kg K_2O/ha and 2.2 kg B/ha from Solubor. The second harvest was taken in August, the third harvest on November 10; and samples for dry matter determination for both harvests were collected from the chopped legume. The percent legume was estimated by four independent observers and the plots measured following each harvest.

In July 1979, whole plants (five/treatment) with intact soil balls were collected from the unlimed plots, 2.3 t/ha hydrated and 13.8 t/ha dolomitic limestone at P treatments 1, 2, and 6. The samples were collected to avoid banded P and were processed as described in the previous study. Soil samples for chemical analysis were collected on six dates: May, July, and September of 1978 and 1979; the number of plots samples was limited due to the large number of sites (210). In May 1978, before liming, samples were collected from all treatments at 0-2, 2-5, 5-10, and 10-20 cm depths. In July 1978, after liming, samples were taken from the top three depths for all treatments. In September 1978, after P treatments, soil samples were collected from the unlimed plots, the high rates of hydrated and dolomitic limestone at P treatments 1, 2, and 6. For the 1979 soil samples, the procedures used in September 1978 were repeated for May and July for the top two soil depths and in September for all four depths.

Figure 2. Application of monoammonium phosphate (11 percent N and 46 percent P_2O_5) with a spray attachment on the Zip seeder.

III. RESULTS AND DISCUSSION

Greenhouse Investigations

Influence of Soil Acidity and Lime on Legume Growth

Three studies were done to examine the effect of lime rates and materials as well as cultural techniques on nodulation, nitrogen fixation, and yield of red clover and/or alfalfa.

Study 1: Response of Alfalfa to Lime. As indicated in the initial soil analysis prior to liming, the levels of Ca and P in the Paxton soil were significantly lower compared to the Agawam and Charlton soils (Table 7). A significant percent of the exchange sites in the Paxton soil were occupied by Al (26 percent). In the Agawam and Charlton soils, Al accounted for 9.6 and 10.0 percent of the exchange sites, respectively. The low Ca and P levels in addition to the high level of Al constitute poor growing conditions for alfalfa in the Paxton soil.

In May, nine months after liming, the soil analysis showed that available P increased with liming in the Charlton soil (Figure 3). In all three soils, Al levels decreased with liming (Table 8). With 4.6 t/ha lime treatment, the Al saturation in the Paxton, Agawam and Charlton soil was 10, 5, and 2 percent, respectively. With an addition of 9.2 t/ha dolomitic lime, the level of Al saturation in the soils was less than 1 percent. Several authors have reported that the soil P response to lime can vary considerably between soils (Lanyon et al., 1977; Mengel and Kamprath, 1978; Reeve and Sumner, 1970a). Reeve and Sumner (1970a)

Table 7. Soil characteristics of the three soils prior to liming. Each value is the mean of three replications.

Soils	pH	Ca	Mg	K	Al	P
		Meq/100 g				ppm
Paxton	5.0 a*	1.06 a	0.35 a	0.29 a	0.59 b	6 a
Charlton	5.0 a	1.82 b	0.36 a	0.28 a	0.28 a	20 b
Agawam	5.0 a	2.27 b	0.30 a	0.26 a	0.30 a	30 c

* Means with a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

Table 8. Nodule number, weight, and percent active nodules (by color) for alfalfa grown in the three soils. Soil Al and pH are also presented. Each value is the mean of four replications.

Lime rate	Soil	pH	Al[1]	Nodule weight per plant	Nodule number per plant	Percent active nodules
t/ha			% of CEC	mg		%
0	Paxton	5.0 a*	26 a	0.2 a	2.2 a	10 a
	Agawam	5.0 a	10 c	2 a	2.2 a	36 b
	Charlton	5.0 a	10 c	3 a	1.8 a	32 b
4.6	Paxton	5.4 b	10 c	31 b	12.0 b	35 b
	Agawam	5.5 b	5 a	55 bc	9.6 b	53 c
	Charlton	5.6 b	2 a	73 c	19.6 c	68 c
9.2	Paxton	6.0 c	1 a	89 cd	25.0 d	82 d
	Agawam	6.3 c	1 a	96 d	19.4 c	86 d
	Charlton	6.2 c	1 a	99 d	23.2 d	83 d

[1] The Al data was arcsin transformed.

* Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Text.

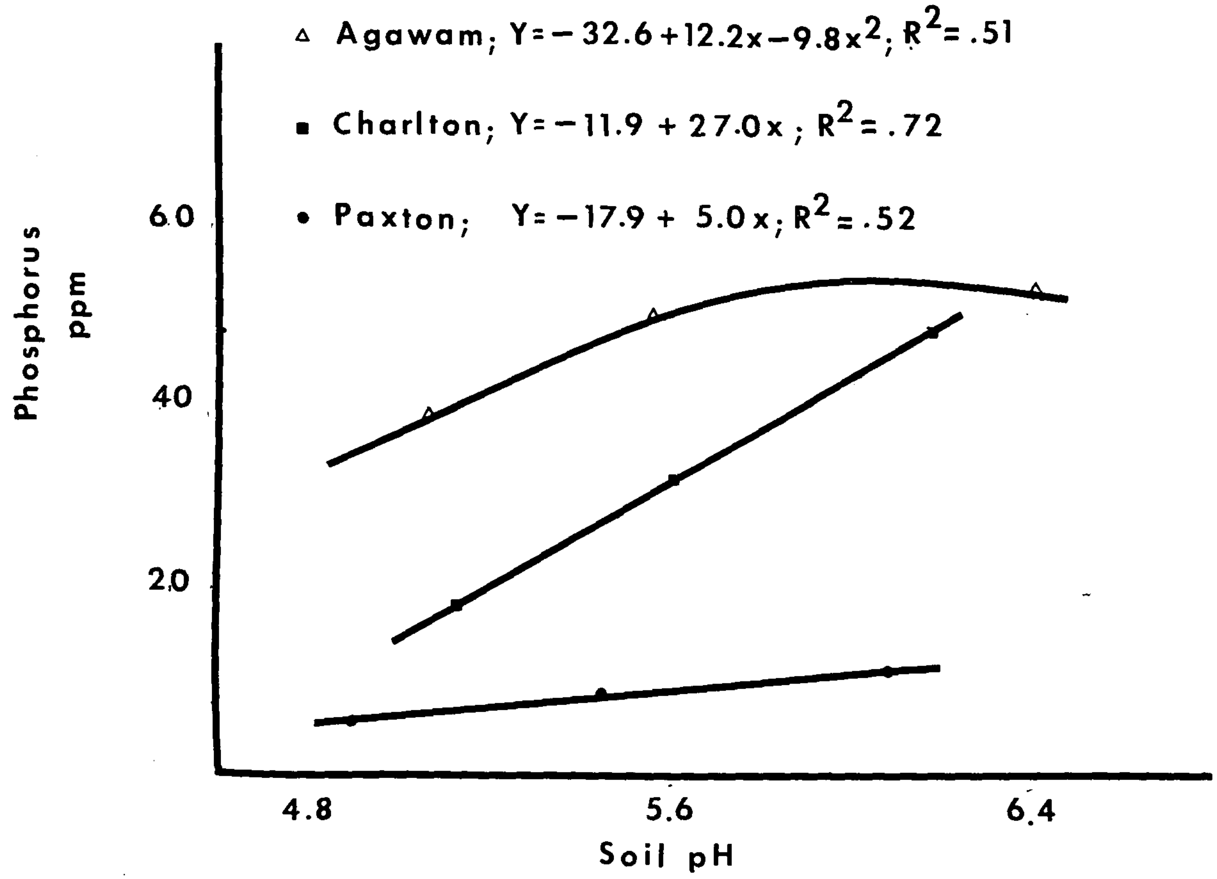


Figure 3. The influence of lime on the availability of P in the soil as shown through regression analysis.

found no relationship between exchangeable Al and P in eight soils: lime had no effect on available P although exchangeable Al levels were reduced.

Total nodule number, nodule weight and percent active nodules increased with lime in the three soils (Table 8). In the Paxton soil, the magnitude of the nodule response to lime was significantly greater than in the other two soils. Alfalfa nodule weight in the Paxton, Agawam, and Charlton soil increased with liming 450, 48, and 33 fold, respectively. The difference in nodule weight of alfalfa in the Paxton soil was probably due to a series of interactions between Al, pH, and P. Nodule number, however, demonstrated a stronger correlation to soil Al than pH or available P (Table 9).

Nitrogen fixation expressed as nM C_2H_4/hr/plant increased for alfalfa in the soils with lime. The manner in which fixation is expressed is an important consideration in evaluating the data (Table 10). When expressed on a per gram of dry weight basis, nitrogen fixation increased 15 to 20 fold following liming and was strongly correlated to soil Al in the three soils (Table 11). On a nodule basis, fixation increased one to two fold.

As indicated in Table 8, nodule weight and the percent active nodules increased with reduced Al saturation; liming produced larger nodules with more active mass. Since nitrogen fixation per milligram of nodule did not increase with lime, the improved nitorgen fixation per plant was not due to higher efficiency (mg N/mg nodule) but to a greater nodule size. Alfalfa yield was significantly correlated to nodule number and weight in the three soils (Table 12). The added lime reduced the Al saturation and brought about some increase in P

Table 9. Regression equations for nodule number with soil pH, aluminum and phosphorus for alfalfa grown in the three soils.

Soil factor	Soil	Regression analysis: Equation	Regression analysis: r^2
[1]pH	Agawam Charlton Paxton	Y = -391 + 85* pH	.69
[2]Al	Agawam	Y = 197 - 2.3* Al	.92
	Charlton	Y = 1223 - 2.3* Al	.81
	Paxton	Y = 138 - 1.5* Al	.92
[2]P	Agawam	Y = 47 + 2.2** P	.43
	Charlton	Y = 9 + 2.2** P	.45
	Paxton	Y = 13 + 9.2** P	.45

[1]Regression equations did not differ significantly at the 0.05 percent level; therefore, pooled form is presented.

[2]Regression equations were significantly different at the 0.05 percent level.

*Significantly different at the 0.01 percent level.
**Significantly different at the 0.05 percent level.

Table 10. Nitrogen fixation for alfalfa expressed in four ways to demonstrate the importance of the manner of presentation in evaluating data. Each value is the mean of four replications.

Lime rate	Soil	Nitrogen fixation per plant	per gram plant dry weight	per nodule	per mg nodule fresh weight
t/ha		nM C_2H_4/hr			
0	Agawam	110 b*	120 b	50 b**	66 c
	Paxton	11 a	28 a	5 a	56 c
	Charlton	85 b	82 b	47 b	33 b
4.6	Agawam	823 b	397 b	86 d	15 a
	Paxton	538 a	301 a	45 b	19 a
	Charlton	882 b	402 b	45 b	14 a
9.2	Agawam	1640 a	602 a	85 d	17 a
	Paxton	1628 a	643 a	65 bcd	18 a
	Charlton	1850 b	749 b	80 cd	19 a

* Means within lime rates in a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

** Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

Table 11. Regression analysis for alfalfa nitrogen fixation with soil aluminum. The data represents three replications per lime rate.

Soil	Nitrogen fixation per gram plant DW Equation	r^2
Agawam	Y = 1578 - 26* Al	.91
Paxton	Y - 1625 - 20* Al	.92
Charlton	Y = 1218 - 25* Al	.91

* Significant at the 0.05 percent level.

Table 12. Regression analysis for alfalfa yield with nodule number (NN) and nodule weight (NW). The data represents three replications per lime rate.

Soil	Equation	r^2
Agawam	Y = 0.12 + 0.0023* NN + 1.03* NW	.91
Charlton	Y = 0.07 + 0.0091* NN + 1.24* NW	.82
Paxton	Y = 0.19 + 0.0100* NN + 1.57* NW	.74

* Significant at the 0.05 percent level.

availability and exchangeable bases. The improved soil fertility improved alfalfa growth providing more energy for nitrogen fixation. According to Hardy and Havelka (1976), photosynthate is the primary limiting factor in the fixation of nitrogen.

Study 2: Response of Alfalfa and Red Clover to Lime Pelleting. At 0 t/ha lime, lime pelleting brought about a significant increase in nodule number and weight for alfalfa. This nodule response to pelleting, however, was not reflected in an improvement of nitrogen fixation or yield (Table 13). Possibly at this soil pH (5.0), inhibition of nitrogen fixation is not just due to reduced nodulation but to other processes; i.e., reduced P translocation to nodules and/or precipitation of nodule P by Al. Sartain and Kamprath (1977) have shown levels of Al in excess of 2000 ppm in soybean nodules and Naidoo et al. (1978) found Al has a major constituent in the nucleus of cotton root cells. With the use of X-ray microanalysis, Naidoo et al. demonstrated Al-P precipitation in membranes. Another possibility is that pelleting provided sufficient Ca for nodulation, but the general low fertility of this Paxton soil (Table 7) interfered in growth.

The lime pelleting had no influence on red clover regardless of the lime rate (Table 13). Clovers, in general, are considered more tolerant of soil acidity than alfalfa. Munns (1965a) found that subterranean clover could form nodules and grow at pH 4.5 while alfalfa was restricted at that pH. Rice et al. (1977) found that red clover nodulation was not significantly improved by liming which was also shown in this experiment. At 0 and 9.2 t/ha dolomitic lime, nodule number for clover averaged 40 per plant. Nitrogen fixation expressed on a

Table 13. Yield, nodule number, weight, and nitrogen fixation for alfalfa and red clover as influenced by broadcast and pelleted dolomitic limestone. Each value is the mean of four replications.

Species	Lime rate	Pelleted	Yield	Nodule number	Weight per nodule	Nitrogen fixation per nodule	per mg nodule
	t/ha		mg/plant	#/plant	mg	nM C_2H_4/hr	
Alfalfa	0	+	22.4 a*	2.5 b	0.95 b	6.5 a	6.8 a
		-	24.4 a	0.6 a	0.40 a	7.1 a	17.8 c
	4.6	+	134.0 b	7.0 c	5.68 c	66.9 b	11.8 b
		-	136.2 b	7.8 c	5.63 c	59.8 b	10.6 b
	9.2	+	348.0 c	12.2 d	4.93 c	165.8 c	34.3 d
		-	353.5 c	11.5 d	5.10 c	151.8 c	29.8 d
Red Clover	0	+	56.0 A**	45.0 A	0.92 A	1.3 A	1.4 A
		-	55.0 A	32.8 A	0.87 A	1.5 A	1.7 A
	4.6	+	169.8 B	40.2 A	0.92 A	10.0 B	10.9 B
		-	194.2 B	48.0 A	1.08 A	8.2 B	7.6 B
	9.2	+	276.2 C	38.5 A	1.00 A	17.5 C	17.5 C
		-	273.8 C	41.5 A	1.00 A	15.8 C	15.8 C

*For alfalfa, means within a column followed by the same small letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

**For red clover, means within a column followed by the same capital letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

nodule basis, increased from 1.4 to 16 nM C_2H_4/hr for red clover while fixation for alfalfa increased from 6.8 to 159.0 nM C_2H_4/hr. At the 9.2 t/ha lime rate, alfalfa nodules were more efficient than red clover, fixing 20 and 16 nM C_2H_4/hr mg nodule, respectively. This difference in nitrogen fixation between the species is probably due to the interaction of several factors; i.e., number of effective nodules per plant, nodule age, effectiveness of specific *Rhizobium* in production of the nitrogenase enzyme, photosynthate supply and/or translocation, etc.

Study 3: Response of Alfalfa to Lime and Superphosphate. In this experiment, the influence of dolomitic limestone, hydrated lime and superphosphate on alfalfa growth was studied. The treatments were applied at five rates without interaction to a soil with an initial pH of 5.6.

The highest rates of dolomitic and hydrated lime increased the soil pH to 6.3 and 7.4 within seven days after application (Table 14). The pH of the soil treated with superphosphate remained at 5.5 to 5.6. The levels of Al in the soil were reduced to approximately 1 ppm with all three treatments at the high rate of application. The most striking difference between the lime and superphosphate was the level of available P in the soil (Table 15). At the highest rate of superphosphate, hydrated and dolomitic limestone, the levels of available P were 21, 1.6, and 2.3 ppm, respectively.

The influence of the differences in soil P on alfalfa weight, nodule number and nodule weight are shown in Table 15. Superphosphate had a stronger influence on nodule number and weight than either of the liming materials. These results clearly demonstrate the positive

Table 14. Soil pH changes at 7, 35, and 54 days after treatment with hydrated, dolomitic lime, and superphosphate.

Treatment	Rate	Days 7	Days 35	Days 54
	t/ha			
Dolomite	0	5.7	5.7	5.5
	2.3	5.8	5.7	5.7
	4.6	5.8	5.8	5.8
	9.2	6.0	6.1	6.1
	18.2	6.3	6.5	6.3
Hydrated	0	5.6	5.6	5.5
	1.2	5.9	5.7	5.6
	2.3	6.3	5.9	5.9
	4.6	6.8	6.6	6.3
	9.2	7.4	7.0	6.0
Superphosphate	0	5.7	5.6	5.5
	0.12	5.5	5.5	5.3
	0.23	5.5	5.5	5.3
	0.46	5.5	5.5	5.4
	0.92	5.4	5.7	5.5

Table 15. The yield, nodule number, and weight per nodule is presented for alfalfa along with soil pH, Al, and P. The soil data was measured on January 3, and the samples were not replicated. All plant characteristics are the means of five replications.

Treatment	Rate	pH	Al	P	Yield per plant	Nodule number per plant	Weight per nodule
	t/ha		ppm		mg		mg
Dolomite	0	5.7	3.0	2.6	100 b*	0.12 a	9.0 c
	2.3	5.7	1.4	2.6	83 ab	1.31 b	2.5 b
	4.6	5.8	1.2	2.5	62 a	0.92 b	2.0 b
	9.2	6.1	1.2	2.3	104 b	3.25 c	2.0 b
	18.4	6.5	1.1	2.3	98 b	0 a	0 a
Hydrated	0	5.6	3.6	3.0	66 a	0.25 a	6.4 ab
	1.2	5.7	2.4	1.9	78 ab	0.45 a	2.9 a
	2.3	5.8	0.9	2.5	63 a	0.50 a	6.8 b
	4.6	6.6	0.9	1.8	91 b	0.20 a	10.5 c
	9.2	7.0	0.9	1.6	84 b	2.00 b	4.0 a
Superphosphate	0	5.6	1.9	2.6	116 ab	3.80 ab	6.3 a
	0.12	5.6	1.8	3.5	96 a	1.80 a	17.0 b
	0.23	5.5	1.4	4.2	206 c	5.20 b	30.8 c
	0.46	5.5	1.4	11.2	196 c	9.70 c	12.9 b
	0.92	5.7	0.9	21.0	128 b	4.35 b	12.0 b

*Within a treatment, means in a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

response of alfalfa to applied P. Munns (1965a, 1965b) showed with hydroponics and in soil that once Al was reduced, alfalfa would respond to fertilizer P. In soil, Munns (1965b) demonstrated that between pH 5.6 and 6.4, the addition of P eliminated the lime response in alfalfa. Jones et al. (1977) have shown with soybeans that nodule number increased with P application.

At the high rate of superphosphate application, plant weight, nodule number, and nodule weight decreased. Tissue analysis did not indicate any significant reductions in the levels of Ca, Mg, Fe, or Zn in the plants due to P application. The levels of P in the tissue averaged 0.18 percent which is lower than expected but total P exceeded 109 mg/plant. The levels of Al increased in the tissue with applied P (Table 16), but there was no indication of Al toxicity. Fletcher and Kurtz (1964) reported a similar growth depression at high levels of applied P for soybeans. At 1323 kg P/ha, the authors reported a substantial decrease in nodule number for the two soybean varieties, Chief and Lincoln. No significant differences were found in tissue levels of Ca, Mg, Fe, Mn, or B. In a paper on Fe deficiencies, Redsike and Biddulph (1954) reported that the distribution of Fe in the leaves is more meaningful than total concentration. Fletcher and Kurtz (1964) indicated that the high levels of P in soybean leaves could bring about conditions favorable for Fe inactivation by the formation of Fe-P precipitates along leaf veins which would not necessarily show up in the total Fe analysis. Levels of plant P available to metabolism could be restricted by precipitation with Al which would decrease growth and nodulation.

Table 16. Composition of alfalfa tissue (tops) following lime or superphosphate treatment. Replications were combined in order to provide sufficent material for analysis.

Treatment	Rate	Ca	Mg	P	Al	Fe	Zn
	t/ha	%			ppm		
Dolomite	0	2.59	0.55	0.09	110	225	41
	2.3	2.80	0.64	0.14	107	150	40
	4.6	1.95	0.52	0.13	45	190	25
	9.2	2.40	0.61	0.12	91	350	25
	18.4	2.55	0.58	0.21	86	275	31
Hydrated	0	2.71	0.60	0.08	121	225	40
	1.2	2.49	0.48	0.09	64	125	21
	2.3	2.33	0.47	0.08	62	125	25
	4.6	4.02	0.57	0.05	34	200	19
	9.2	4.21	0.74	0.11	48	225	19
Superphosphate	0	2.62	0.65	0.14	48	175	39
	0.12	2.68	0.76	0.13	48	150	39
	0.23	2.81	0.53	0.15	54	125	37
	0.46	2.51	0.72	0.18	194	400	31
	0.92	2.58	0.69	0.17	123	275	40

Effects of Aluminum on Aeroponically Grown Alfalfa and Red Clover

Aluminum stress was greater for the plants totally dependent on nitrogen fixation. The addition of Al at either 1 or 2 ppm brought about significant reductions in nitrogen fixation; by the fourth week, the decrease in fixation at 1, 2, 2 ppm Al + N was 33, 72, and 94 percent, respectively (Table 17).

Table 17. Nitrogen fixation of alfalfa at 1, 2, and 2 ppm Al + N expressed as a percent of the activity at 0 ppm Al (100%).

Treatment	Weeks 1	2	3	4
		%		
0 ppm Al	100	100	100	100
1 ppm Al	100	75	69	67
2 ppm Al	97	76	47	28
2 ppm Al + N	55	23	11	6

Even though the added N severely restricted nitrogen fixation, plant yield was not significantly less than those at 0 ppm Al (Table 18). These data indicate that at these concentrations, Al toxicity is primarily on the fixation mechanisms. Andrews (1977) in a review paper suggested that the primary damage of Al on sensitive legumes is on nodulation and nitrogen fixation. When fertilizer N is present, the damaged symbiosis loses its inhibition on the growth of the host

Table 18. Effects of aluminum on the yield, nodule number, nodule weight, and nitrogen fixation of alfalfa grown aeroponically. Each value is the mean of four replications.

Week	Treatment	Yield	Nodule number per plant	Weight per plant	Nitrogen fixation
		mg/plant		mg	nM C_2H_4/hr/nodule
1	0 ppm Al	8.6 a*	10.6 abc	1.2 ef	80.1 c
	1 ppm Al	8.2 a	9.8 abc	0.9 de	90.3 c
	2 ppm Al	6.0 a	9.0 abc	0.7 cd	78.3 c
	2 ppm Al +N	8.0 a	7.4 a	0.6 bcd	43.8 b
2	0 ppm Al	37.2 b	11.8 bcd	2.0 h	157.8 e
	1 ppm Al	34.0 b	12.3 cd	0.9 de	118.6 d
	2 ppm Al	28.6 b	9.2 abc	0.5 abc	119.4 b
	2 ppm Al + N	36.6 b	8.8 ab	0.3 ab	36.8 a
3	0 ppm Al	71.2 d	14.3 de	2.5 h	203.5 f
	1 ppm Al	59.9 c	9.8 abc	1.0 ef	140.8 de
	2 ppm Al	37.2 b	9.5 abc	0.7 cd	95.5 c
	2 ppm Al + N	72.0 d	7.4 a	0.2 a	21.9 a
4	0 ppm Al	102.0 e	16.9 e	2.9 h	299.4 g
	1 ppm Al	74.4 d	11.4 bcd	1.3 g	204.1 f
	2 ppm Al	48.4 c	10.6 abc	0.4 a	84.4 c
	2 ppm Al + N	104.4 e	10.0 abc	0.1 a	17.8 a

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

plant. Munns (1965a) demonstrated that N could reduce problems of soil acidity if Al levels were low. Munns (1965b) refers to the formation of Al-P colloidal compounds in solution that are not clearly visible and that such formations reduce Al levels in solution. There is no clear way of knowing if such compounds did form in this experiment.

Harper and Hageman (1972) found that added N severely restricted nitrogen fixation in soybeans grown hydroponically. The authors indicated that the reduction in fixation was significantly correlated to nodule number. In this experiment, the plants were already nodulated and fixing nitrogen when N was added to the nutrient solution. Nodule weight, however, significantly decreased in the plants exposed to N (Table 18). Munns (1977) stated that fertilizer N retards nodule growth and in its presence, the nodules contain less legahemoglobin and receive less photosynthate from the shoot.

The addition of the Al to the nutrient solution had a less potent influence on red clover compared to alfalfa (Tables 19 and 20). The reduction in nitrogen fixation in the fourth week for 1, 2, 2 ppm Al + N was 10, 16, and 96 percent, respectively. Nodule weight in red clover was also reduced by the Al, but the inhibition in nodule growth and nitrogen fixation was not reflected in yield (Table 20). Red clover yields were not reduced by the presence of Al. The percent reduction in red clover nitrogen fixation in the fourth week at 2 ppm Al was 16 percent which is small compared to the 72 percent reduction in fixation for alfalfa.

Table 19. Nitrogen fixation for red clover at 1, 2, 2 ppm Al + N expressed as a percent of the activity at 0 ppm Al (100%).

Treatment	Weeks 1	2	3	4
		%		
0 ppm Al	100	100	100	100
1 ppm Al	100	88	95	10
2 ppm Al	100	92	97	84
2 ppm Al + N	100	37	10	4

Effects of Fertilizer Nitrogen on Nitrogen Fixation and Nitrate Reduction in Alfalfa and Red Clover

In this experiment, alfalfa and red clover were grown at 0 and 60 kg N/ha in a soil of pH 5.6. Plants were harvested weekly for two months for yield, nitrogen fixation, nodulation, and nitrate reduction.

For both legumes, the application of N severely restricted nitrogen fixation (Figures 4 and 5) without reducing yield (Tables 21 and 22). Alfalfa is known to acquire 60 to 90 percent of its nitrogen via fixation while the figures for red clover range from 20 to 40 percent (Vance, 1978). This relative relationship is reflected in the lower fixation of clover compared to alfalfa in the soil without N (Figures 4 and 5). Nitrate reductase activity in clover was two to five times the rate in alfalfa which indicates that red clover is more dependent on soil nitrate for its N supply.

Table 20. Effects of aluminum on the yield, nodule number, nodule weight, and nitrogen fixation of red clover grown aeroponically. Each value is the mean of four replications.

Week	Treatment	Yield	Nodule number per plant	Weight per nodule	Nitrogen fixation
		mg/plant		mg	nM C_2H_4/hr/nodule
1	0 ppm Al	15.6 a*	24.9 a	0.30 b	14.5 b
	1 ppm Al	17.2 a	35.9 a	0.33 b	15.1 b
	2 ppm Al	16.6 a	36.0 a	0.30 b	16.5 b
	2 ppm Al + N	14.6 a	38.1 a	0.26 a	14.2 b
2	0 ppm Al	37.8 b	34.4 a	0.41 c	30.4 c
	1 ppm Al	42.0 b	34.9 a	0.51 d	26.8 c
	2 ppm Al	35.2 b	41.5 a	0.44 cd	28.1 b
	2 ppm Al + N	38.4 b	40.4 a	0.40 c	10.6 b
3	0 ppm Al	85.4 c	37.3 a	1.00 g	42.1 d
	1 ppm Al	96.4 c	38.3 a	0.76 f	40.5 d
	2 ppm Al	87.0 c	38.1 a	0.64 e	40.8 d
	2 ppm Al + N	80.0 c	39.2 a	0.33 b	4.0 ab
4	0 ppm Al	124.6 d	20.8 a	1.13 g	57.0 e
	1 ppm Al	122.0 d	33.9 a	1.12 g	51.0 e
	2 ppm Al	122.0 d	36. 9 a	0.63 e	48.3 de
	2 ppm Al + N	129.0 d	42.1 a	0.21 a	2.0 a

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

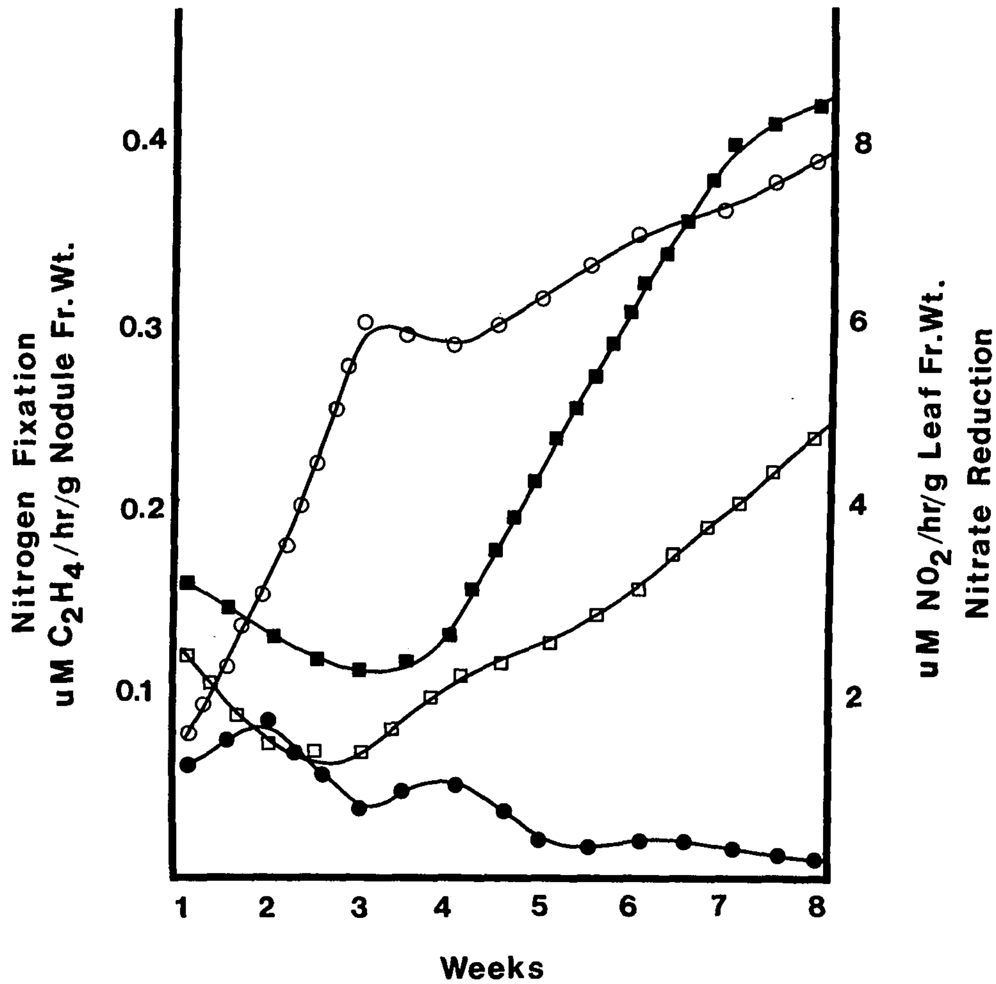


Figure 4. Influence of fertilizer nitrogen on nitrogen fixation and nitrate reduction in red clover. Nitrogen fixation at 0 kg N/ha ○, at 60 kg N/ha ●. Nitrate reduction at 0 kg N/ha □, at 60 kg N/ha ■.

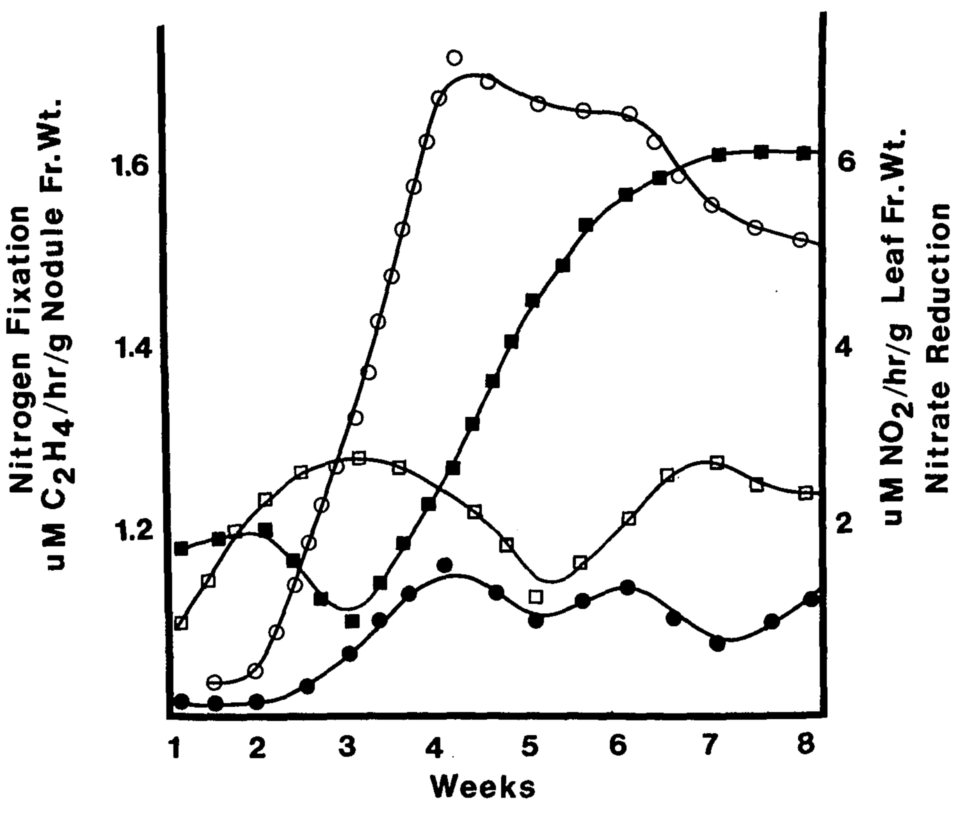


Figure 5. Influence of fertilizer nitrogen on nitrogen fixation and nitrate reduction in alfalfa. Nitrogen fixation at 0 kg N/ha ○, at 60 kg N/ha ●. Nitrate reduction at 0 kg N/ha □, at 60 kg N/ha ■.

Table 21. Influence of nitrogen fertilizer on the yield, nodule number, and weight per nodule for alfalfa over an eight-week period. Each value is the mean of three replications.

Date	Nitrogen	Yield per plant	Nodule number per plant	Weight per nodule
		mg		mg
4/11	-	46.8 a*	12.0 a	0.8 abc
	+	50.8 a	12.0 a	0.4 a
4/18	-	71.4 a	11.9 a	1.3 cd
	+	61.6 a	12.4 a	0.5 ab
4/25	-	135.0 bc	11.1 a	1.9 e
	+	111.6 b	11.1 a	1.0 bcd
5/2	-	170.8 d	11.5 a	2.6 f
	+	160.4 cd	11.8 a	0.6 a
5/9	-	327.4 ef	12.5 a	1.9 e
	+	299.6 e	12.2 a	1.4 de
5/16	-	372.6 h	11.6 a	4.3 g
	+	360.4 gh	11.3 a	1.5 de
5/24	-	360.0 gh	11.7 a	2.5 f
	+	346.2 fgh	11.0 a	1.0 bcd
5/30	-	361.0 gh	11.9 a	2.8 f
	+	341.8 fg	10.3 a	1.3 cd

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

Table 22. Influence of nitrogen fertilizer on red clover yield, nodule number, and weight per nodule over an eight-week period. Each value is the mean of three replications.

Date	Nitrogen	Yield per plant	Nodule number per plant	Weight per nodule
		mg		mg
4/11	-	35.6 a*	13.4 abc	0.6 abc
	+	22.0 a	11.6 a	0.9 cd
4/18	-	58.6 a	14.5 abc	0.4 a
	+	55.4 a	13.1 abc	0.8 bc
4/25	-	129.2 b	11.6 a	0.8 bc
	+	132.6 b	12.1 ab	0.9 cd
5/2	-	196.6 c	15.4 c	0.5 ab
	+	182.4 c	14.2 abc	0.5 ab
5/9	-	384.8 de	15.1 bc	0.6 abc
	+	376.6 d	14.9 bc	0.4 a
5/16	-	404.8 ef	19.5 de	0.6 abc
	+	381.3 de	19.1 d	0.5 ab
5/24	-	412.2 f	24.3 fg	0.9 cd
	+	413.8 f	22.6 ef	0.6 abc
5/30	-	419.6 f	26.1 g	1.2 d
	+	416.2 f	32.0 h	0.5 ab

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

Nodule weight in alfalfa was significantly reduced by the addition of N; in the fourth week, the weight per nodule for alfalfa grown at 0 and 60 kg N/ha was 2.5 and 0.6 mg, respectively. Chen and Phillips (1977) reported nodule senescence in garden pea when nitrate was added to the soil. Clover nodules were also reduced in weight, but the percent reduction was much less than for alfalfa. Alfalfa may be more dependent on soil nitrate at pH 5.6 since growing conditions are not considered optimal for nodule growth (Munns, 1965a). Previous experiments have shown that the reduction of Al levels and the increase in P are more critical than reaching a pH of 6.0. The soil used in this experiment had less than 4 ppm Al, and the level of P exceeded 25 ppm which is considered a medium to high level (Olsen and Dean, 1969).

Several authors have shown severe inhibition of nodulation in alfalfa by soil N (Heichel and Vance, 1977; Munns, 1968a; Thorton, 1936; Vance, 1978). Heichel and Vance demonstrated a 40 percent reduction in nodule number at 50 ppm N, and Munns found that as little as 3 ppm N in solution reduced nodule number by 90 percent. The mechanism of nitrate interference in nodulation is not completely understood. Chen and Phillips (1977) demonstrated that nitrate supplied to the leaves of garden pea did not cause nodule senescence which indicates that nitrate does not bring about nodule inhibition by competing with fixation for photosynthate. Munns (1968b, 1977) suggested that the Rhizobium converts nitrate to nitrite which, in turn, inhibits production of the IAA required in the infection process.

The increase in nitrate reduction at the expense of nitrogen fixation suggests a preference for nitrate as the N supply. In this experiment, the availability of soil nitrate brought about a 60 percent increase in nitrate reductase activity in alfalfa and a 30 percent increase in red clover. Gibson (1977) indicates that both nitrate assimilation and nitrogen fixation require similar amounts of energy. Silsbury (1977) demonstrated that the energy involved in infection, nodule growth, translocation and synthesis in nitrogen fixation is much more of an energy drain on the host plant than nitrate assimilation.

Alfalfa and red clover yields were not significantly increased by the applied N (Tables 21 and 22). Similar results have been shown for a variety of legumes (Hardy and Havelka, 1976; Lawn and Brun, 1974; Harper, 1974). This ineffectiveness of applied N has been attributed to the compensatory decrease in nitrogen fixation that approximates the increase in nitrate assimilation due to the fertilizer N. Hardy and Havelka (1976) found that an increase in ambient CO_2 levels in the atmosphere brought about five to six fold increases in nitrogen fixation and doubled the yield of soybeans. This indicates that for nitrogen fixation, the limiting factor is the supply of photosynthate to the nodules.

Influence of Soil Amendments and Rhizobium Strain on the Nodulation of Alfalfa and Red Clover

Two experiments were conducted on the relationship of soil acidity and the effectiveness of *Rhizobium* strains to the nodulation of red clover and/or alfalfa.

Study 1: Interactions of Rhizobium meliloti Strain, Alfalfa Variety and Soil pH. The analysis of variance indicated that significant interactions existed between strain, variety and pH that had a significant influence on nitrogen fixation (Table 23). The data showed that alfalfa inoculated with R. meliloti strains F51 and F77 had the lowest nitrogen fixation rates regardless of variety or soil pH (Table 24). At soil pH 5.0, nitrogen fixation rates ranged from 10 to 134 nM C_2H_4/hr/plants; Northrup King (NK) alfalfa inoculated with strain F66 had the highest rate. Alfalfa inoculated with R. meliloti strains F52, F66, and F70 demonstrated nitrogen fixation rates in excess of 450 nM C_2H_4/hr/plant at soil pH 5.5. Saranac alfalfa inoculated with strain F52 and Arc alfalfa inoculated with strain F70 had nitrogen fixation rates at pH 6.0 of 1512 and 1318 nM C_2H_4/hr/plant, respectively. The nitrogen fixation data showed that no one combination of R. meliloti strain and alfalfa variety was the best match at all pH levels. Alfalfa inoculated with strains F52, F66, and F70, however, demonstrated the highest fixation rates of the strains tested.

The plants with the highest fixation rates had the most nodules (Table 25). Northrup King alfalfa inoculated with strain F66 averaged 19.7 nodules per plant at pH 5.0 and 23.0 nodules per plant at pH 5.5. At pH 6.0, Arc alfalfa inoculated with strain F70 and Saranac alfalfa inoculated with strain F52 had 23.7 and 20.0 nodules per plant, respectively.

Several studies have shown that nitrogen fixation is optimal when the most effective bacteria is combined with the best plant variety (Bolton, 1972; Gibson, 1962; Purchase and Nutman, 1957). Bolton indicated that nitrogen fixation can vary from less than 2.0 mg N to over 5 mg N/plant/day. Seetin and Barner (1977) tested the nitrogen fixation of ten different alfalfa clones via acetylene

Table 23. The F ratios from the analysis of variance for Rhizobium meliloti strain, alfalfa variety, and soil pH. Data are from Greenhouse Experiment.

Source	Df	Yield	Nodule number	Nitrogen fixation
Strain (S)	6	3.79 **	29.1 **	132.7 **
Variety (V)	2	5.70 **	3.8 **	22.4 **
pH (P)	2	84.92 **	164.6 **	1804.5 **
S x V	12	ns	5.3 **	25.5 **
S x P	12	ns	4.9 **	36.8 **
V x P	4	ns	ns	14.1 **
S x V x P	24	ns	3.3 *	16.9 **

*significant at the 0.05 percent level.

**significant at the 0.01 percent level.

ns not significant

Table 24. Rates of nitrogen fixation for three varieties of alfalfa inoculated with the seven strains of Rhizobium meliloti at pH 5.0, 5.5, and 6.0. Each value is the mean of three replications.

pH	Variety	Strains 51	52	66	70	73	77	82
		uM C_2H_4/hr/plant						
5.0	Arc Al-14	10 aA*	17 aA	56 aC	95 bE	77 cD	36 aB	39 aB
	Saranac	50 bCD	24 aA	73 bE	44 aC	18 aA	34 aB	59 bD
	NK7-5	43 bD	56 bCD	134 cE	47 aBC	42 bB	33 aA	64 bD
5.5	Arc Al-14	134 aA	230 aB	296 aBC	531 aD	330 bC	73 aA	340 bC
	Saranac	215 bB	578 cD	302 aB	599 aD	256 bB	82 aA	434 cC
	NK7-5	212 bA	496 bB	499 cB	537 aB	156 aA	206 bA	256 aA
6.0	Arc Al-14	375 aA	689 aC	745 bCD	1318 bE	528 bB	283 aA	850 bD
	Saranac	548 bB	1512 bD	727 aC	752 aC	256 aA	598 bB	599 aB
	NK7-5	660 bBC	745 aCD	452 aAB	870 aD	456 bB	300 aA	764 bCD

* Within each pH, means in a column followed by the same small letter and means in a row followed by the same capital letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

Table 25. Nodule number for the three varieties of alfalfa inoculated by the seven strains of Rhizobium meliloti at pH 5.0, 5.5, and 6.0. Each value is the mean of three replications.

pH	Variety	Strains						
		51	52	66	70	73	77	82
		nodule number/plant						
5.0	Arc Al-14	3.7 bAB*	3.7 aAB	5.7 aC	9.7 bD	4.0 bB	6.7 bC	2.7 aA
	Saranac	2.3 aA	5.3 bC	9.0 bD	4.0 aB	2.0 aA	2.3 aA	3.7 aB
	NK7-5	1.3 aA	3.3 aB	19.7 cD	6.0 aC	3.3 bB	2.7 aB	6.0 bC
5.5	Arc Al-14	7.0 aA	10.7 aC	9.3 aB	16.0 bD	9.0 bB	7.0 bA	11.0 bC
	Saranac	7.0 aB	16.0 bD	11.3 aC	17.7 bD	6.3 aAB	4.3 aA	10.7 bC
	NK7-5	10.6 bB	19.3 cC	23.0 bC	14.0 aB	10.3 bB	5.1 abA	5.7 aA
6.0	Arc Al-14	11.0 aB	15.3 aC	12.0 aB	24.0 bD	11.7 aB	5.0 aA	16.0 bC
	Saranac	8.3 aA	20.0 bE	14.0 aD	10.3 aB	10.7 aBC	11.7 bC	9.3 aAB
	NK7-5	10.0 aB	23.0 bD	23.0 bD	12.7 aC	11.0 aBC	6.7 aA	11.0 aBC

* Within each pH, means in a column followed by the same small letter and means in a row followed by the same capital letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

reduction and found a range of fixation from 0.47 to 2.09 uM C_2H_4/hr/plant. According to Vance (1978), the difference in fixation is due to variation in the bacteria and the host plant.

Alfalfa yields were significantly influenced by R. meliloti strain, alfalfa variety and soil pH (Table 26). All Rhizobium strain-varieties that gave high fixation rates demonstrated high yields but not all the high yields were from such a combination. At pH 5.0, four combinations of strain and variety had yields that exceeded 165 mg per plant, but only one of these combinations demonstrated high nitrogen fixation rates; i.e., NK alfalfa inoculated with strain F66. The data indicates that other factors affect yield in alfalfa besides nitrogen fixation. The soil analysis indicated medium levels of nitrogen which may have played a more important role in growth where nitrogen fixation rates were low.

Although some of the strain-variety interactions led to significantly higher nitrogen fixation rates and yields, there is no guarantee that the combination would give similar results in the field. According to Evans (1975) little success has been obtained in establishing introduced Rhizobium in the field with high native populations. The introduced strains cannot compete with the well established native populations. Evans states that methods must be found to enhance the competition of selected strains from laboratory and greenhouse studies or these strains will not be able to be utilized. Keyser and Munns (1979 a, 1979b) and Keyser et al. (1979) selected strains of cowpea and soybean Rhizobium from culture that were tolerant to soil Al and acidity. The authors indicated that the strains tolerant in culture

Table 26. Yield of the three varieties of alfalfa inoculated with the seven strains of Rhizobium meliloti at pH 5.0, 5.5, and 6.0. Each value is the mean of three replications.

pH	Variety	Strains 51	52	66	70	73	77	82
					mg/plant			
5.0	Arc Al-14	141 abA*	140 abA	134 aA	189 bB	144 aA	142 aA	158 aA
	Saranac	174 bB	152 bAB	168 bB	135 aA	149 aAB	156 aAB	129 aA
	NK7-5	111 aA	117 aA	167 bC	154 aBC	131 aAB	133 aAB	130 aAB
5.5	Arc Al-14	244 bBC	195 abA	218 aAB	235 bBC	245 aC	211 abAB	228 bABC
	Saranac	168 aA	227 bBC	256 abC	204 bB	224 aB	174 aA	208 abB
	NK7-5	140 aA	186 aBC	288 bE	167 aAB	203 aCD	225 bD	180 aBC
6.0	Arc Al-14	212 aA	346 aB	343 aB	380 aB	399 bB	337 aB	335 aB
	Saranac	249 aA	508 cD	363 aBC	422 aC	384 abB	359 aB	323 aB
	NK7-5	212 aA	439 bD	410 aD	413 aD	310 aBC	371 aCD	281 aAB

* Within each pH, means in a column followed by the same small letter and means in a row followed by the same capital letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

did not always correspond to the results in the field. Strain competition was cited as one source of the problem.

Study 2: Influence of Applied Lime and Phosphorus and the Presence of the Host Plant on the Effectiveness of Rhizobium in the Nodulation of Alfalfa and Red Clover. In this experiment, alfalfa and red clover were sown in both the inoculated and uninoculated condition into soil collected from two field experiments - the Harmony Hill Farm experiment with dolomitic limestone and the Demerit experiment with dolomitic and hydrated limestone in combination with superphosphate treatments. A modified version of a scoring system of Rice et al. (1977) which includes nodule color, position, number, and size was used to measure nodulation (Table 27).

This nodule scoring system presents several advantages over the recording of nodule weight and number. The system takes into account four variables in nodulation patterns and balances them together into one result. The scoring system allows for rapid estimation of the nodulation pattern without the painstaking method of nodule removal, counting, and weighing.

Seeding date and inoculation had a significant influence on the nodulation score of alfalfa seeded in the soil from the Harmony Hill Farm (Table 28). The older the seeding, the less influence the inoculant had on the nodulation of alfalfa. Alfalfa grown in the soil taken from the May 1978 seeding showed scores of 9.6 and 9.2 for inoculated and uninoculated plants. The two factors that changed for alfalfa were nodule color and size; the plants sown into the soil taken from the May 1978 seeding date had larger and more active nodules.

Table 27. Brief description of the nodule scoring system used to evaluate nodulation in alfalfa and red clover.[1]

Nodule factor	Highest score	Description
Color	4	percent pink nodules
Position	4	percent of the total nodules within 5 cm of the crown
Number	5	total nodule number
Size	3	nodule diameter

[1]For a more complete description of the scoring system, see Table 4.

Red clover sown into the soil from the Harmony Hill Farm behaved differently than the alfalfa. The uninoculated plants gave higher scores than the inoculated plants, and this difference was associated with seeding date (Table 29). For red clover grown in the soil from the May 1978 seeding date, the nodulation score for inoculated and uninoculated plants was 2.2 and 4.2, respectively. The nodulation score for clover in soil from the May 1979 seeding date for inoculated and uninoculated was 3.9 and 4.2, respectively. The data indicates that the effectiveness of the inoculant for red clover nodulation decreased with the age of the seeding. Breaking down the nodulation score into component parts indicated that the two factors differing between inoculated and uninoculated plants were nodule color and number. The uninoculated plants had more nodules, but the majority

Table 28. Nodulation score of alfalfa seeded into the soil collected from Harmony Hill Farm as influenced by lime and inoculated treatment. Each value is the mean of five replications.

Seeding date	Lime rate	Incoulant	Nodulation Score Color	Position	Number	Size	Total
	t/ha		units				
May 1979	4.6	+	2.0	0.7	1.5	0.5	4.7 ab*
		-	0.3	0.8	2.1	0.1	3.0 a
	13.8	+	2.7	1.5	2.1	0.6	6.9 cde
		-	0.4	1.0	1.2	0.6	3.2 a
August 1978	4.6	+	3.2	1.1	1.6	1.5	6.4 cd
		-	0.8	0.8	1.8	1.0	4.4 ab
	13.8	+	3.6	1.5	1.3	1.0	7.4 def
		-	1.7	1.0	1.1	1.5	5.3 bc
May 1978	4.6	+	3.2	2.5	1.8	1.8	9.4 fgh
		-	3.1	2.1	2.1	1.2	8.7 efg
	13.8	+	4.1	2.9	2.1	1.2	10.0 h
		-	3.0	2.6	2.6	1.4	9.6 gh

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

Table 29. Nodulation score for red clover seeded into the Harmony Hill soil as influenced by lime and inoculation treatments. Each value is the mean of five replications.

Seeding date	Lime rate	Inoculant	Nodulation Score Color	Position	Number	Size	Total
	t/ha		units				
May 1978	4.6	+	0.34	0.86	1.7	0	2.7 ab*
		-	0.06	0.80	3.2	0.06	4.1 c
	13.8	+	0.44	1.00	0.2	0	1.7 a
		-	0.06	1.26	2.9	0	4.2 c
August 1978	4.6	+	0.14	1.94	2.4	0	4.5 c
		-	0	3.10	3.3	0	6.4 e
	13.8	+	0.80	1.06	1.7	0.14	3.7 bc
		-	0.02	0.80	4.2	0.92	5.9 de
May 1979	4.6	+	0.26	1.46	1.3	0	3.2 bc
		-	0.40	1.88	1.6	0	3.9 bc
	13.8	+	0	1.98	3.3	0	4.6 cd
		-	0.70	1.74	2.6	0	4.5 cd

*Means within a column followed by the same small letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

of them were white. According to Jordan (1974), the presence of numerous small white nodules indicates nodulation by ineffective strains of Rhizobium. A possible explanation for the increase of these ineffective infections with seeding date is the sensitivity of R. trifolii to amensalism especially from soil fungi (Parker et al., 1977). Diagnosis by the personnel of the plant pathology department indicated a severe infection of Fusarium solani on the red clover, and field observations indicated that this infection was worst for plants seeded in May 1978. Jordan (1974) indicated that environmental stress and/or disease could bring about transient ineffectiveness in Rhizobium.

The high nodulation score for the uninoculated alfalfa grown in the soil from the May 1978 seeding date is probably due to the encouragement of populations of Rhizobium meliloti introduced in the inoculant from the original seeding. The stimulation of Rhizobium populations in the soil by legumes has been discussed by Vincent (1965). The Rhizobium often appear poorly adapted to survival in the soil in the absence of the host plant and will quickly dwindle in number if the legume is removed (Parker et al., 1977). Nutman (1963) showed that legumes secrete substances from their roots which cause Rhizobium to multiply in the area of the plant roots.

The scanning electron micrographs of alfalfa nodules did not show any morphological changes that were correlated to lime rate. The micrographs of nodules taken from alfalfa grown at 4.6 and 13.8 t/ha dolomitic limestone show nodule cells with varying numbers of R. meliloti (Figures 6 and 7), but there is no consistent cell volume of bacteria that corresponded to pH. The majority of the bacteria in the cells are swollen

Figure 6. Scanning electron micrographs of alfalfa nodules taken from a plant grown with 4.6 t/ha dolomitic limestone.

A. Exterior view of nodule

Conditions:

magnification 60x
tilt 15.5^{o}
20 k.v.

B. Longitudinal section showing exposed nodule cells

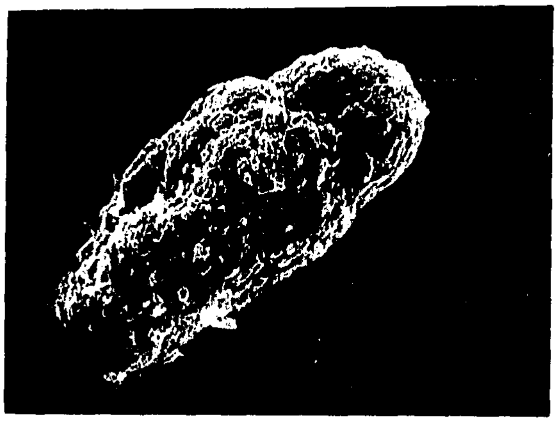
Conditions:

magnification 600x
tilt 15.5^{o}
20 k.v.

C. Closeup of a single nodule cell showing bacteroids

Conditions:

magnification 2200x
tilt 15.5^{o}
20 k.v.



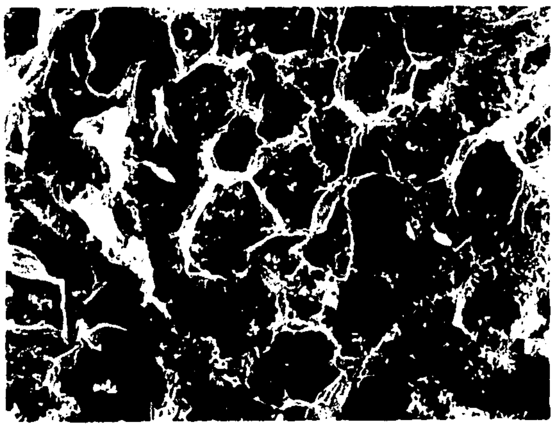


Figure 7. Scanning electron micrographs of alfalfa nodules taken from a plant grown with 13.8 t/ha dolomitic limestone.

A. Exterior view of the nodule

Conditions:

magnification 60x
tilt 15.5°
20 k.v.

B. Longitudinal section showing exposed nodule cells

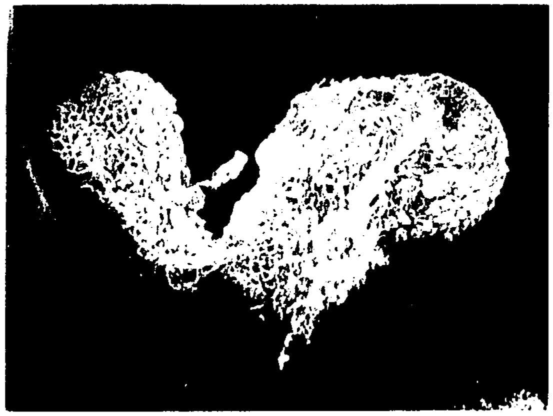
Conditions:

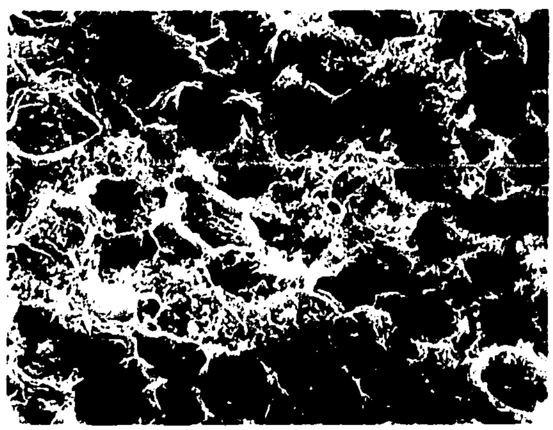
magnification 600x
tilt 15.5°
20 k.v.

C. Closeup of a single nodule cell showing some bacteroids

Conditions:

magnification 2200x
tilt 15.5°
20 k.v.





rods called bacteriods, and it is within these forms that nitrogenase occurs (Bergensen, 1977). Micrographs of red clover nodules also did not demonstrate any significant correlation to liming, but bacteria in the nodule cells of clover were quite different from those in alfalfa (Figure 8). The nodules of red clover were small and white, and the R. trifolii within the cells were round with very few rods in evidence. Bergensen (1970) stated that naturally occurring bacteria produced nodules which contained a relatively small amount of bacteriod tissue, and there was considerable volume of degenerated tissue in each nodule.

The results for alfalfa grown in the soil from the Demerit experiment indicated that nodulation was influenced differently by inoculation, lime, and P treatments (Table 30). With dolomitic limestone, inoculation had no significant influence on nodulation regardless of the level of applied P. These data indicate that the populations of Rhibozium meliloti in the soil were as effective in nodulating alfalfa as the introduced strains in the inoculant. The nodulation score for alfalfa increased with the levels of applied P such that the scores at 0, 220, and 440 kg P_2O_5/ha averaged for inoculated and uninoculated plants were 8,0, 9.2, and 10.8, respectively. This increase in the nodulation score was due to an improvement in nodule color and size.

With hydrated limestone, uninoculated alfalfa had significantly higher nodulation score at 0 and 220 kg P_2O_5/ha but at 440 kg P_2O_5/ha inoculation did not influence nodulation (Table 30). The hydrated lime reduced the effect of the introduced Rhizobium meliloti, but the inhibition of nodulation was lessened by the increased application of P. At 440 kg P_2O_5/ha, both inoculated and uninoculated plants had

Figure 8. Scanning electron micrographs of alfalfa and red clover nodules.

A. High magnification of a single cell from alfalfa nodule showing bacteroids

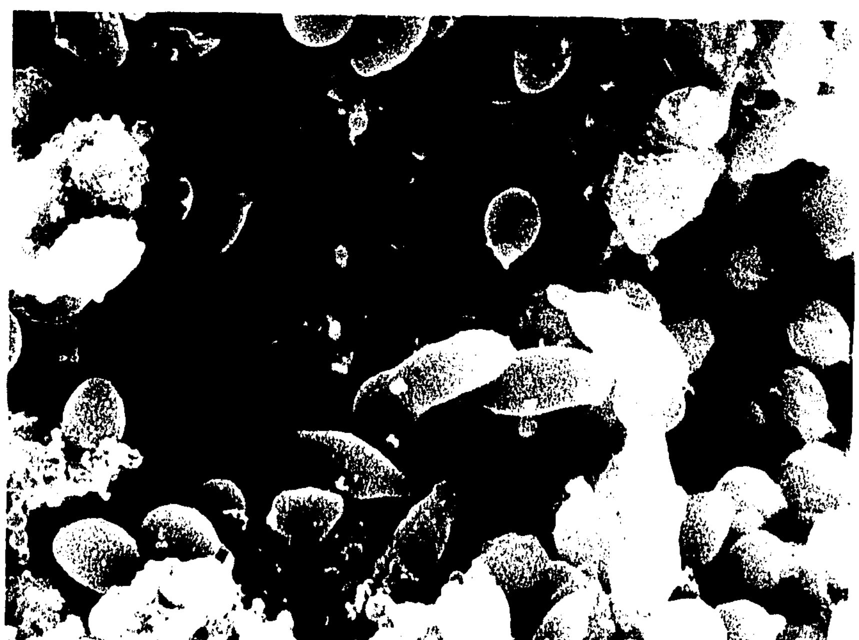
Conditions

magnification 5000x
tilt 15.5^{o}
20 k.v.

B. High magnification of a single cell from red clover nodule showing odd-shaped Rhizobium

Conditions:

magnification 5600x
tilt 15.5^{o}
20 k.v.



nodulation scores of 6.5 and 7.2, respectively, while at 0 kg P_2O_5/ha, the scores were 4.0 and 10.2, respectively. The mechanism of adaptation of R. meliloti soil populations to the hydrated lime is not known, but under stress conditions native populations have shown greater survival than introduced Rhizobium (Parker et al., 1977).

In the soil that was unlimed, P addition significantly influenced nodulation, but inoculation had no benefit (Table 30). At 0, 220, and 440 kg P_2O_5/ha, the nodulation score for alfalfa averaged over inoculated and uninoculated plants was 7.4, 10.4, and 11.1, respectively. The increase in the nodulation score was primarily due to changes in nodule color, size and number. From 0 to 220 kg P_2O_5/ha, nodule number, size, and color increased 28, 69, and 30 percent, respectively.

The nodulation of alfalfa grown in the soil that received dolomitic lime was not significantly different from that in the unlimed soil. This indicates that soil pH was not important in this soil for alfalfa nodulation. The data also demonstrates that for plants in the dolomitic lime and unlimed soil, the level of applied P had a significant influence on nodulation but inoculation showed no benefit. The added P improved the environment for Rhizobium meliloti populations. This is clearly shown where alfalfa was seeded into untreated soil collected from the border of the experiment; the nodulation score for inoculated and uninoculated plants was 7.6 and 2.0, respectively.

The nodulation of red clover sown into the soil that received hydrated limestone was significantly less than the score in the unlimed or dolomitic lime soil (Table 31). The nodulation score of red clover averaging inoculated and uninoculated plants in unlimed, dolomitic lime

Table 30. Nodulation score for alfalfa seeded into the soil collected from the Demerit site as influenced by phosphorus and inoculation treatments. Each value is the mean of five replications.

Lime treatment	Phosphorus rate	Inoculation	Nodulation Score Color	Position	Number	Size	Total
	kg P_2O_6/ha		units				
Dolomite	0	+	2.0	1.7	3.7	1.0	8.3d*
		-	2.0	1.6	3.8	1.0	8.3 d
	220	+	3.5	1.5	3.9	0.4	9.3 e
		-	3.0	2.0	3.7	0.4	9.1 e
	440	+	3.7	1.8	4.6	0.8	10.9 f
		-	4.1	2.0	3.9	1.1	11.1 f
No lime	0	+	2.7	1.2	3.2	0.3	7.4 cd
		-	3.0	1.5	3.3	0.3	8.1 d
	220	+	3.7	2.4	3.4	0.9	10.4 f
		-	4.1	2.2	3.8	1.1	11.0 f
	440	+	3.8	1.9	4.0	1.4	11.1 f
		-	3.2	2.1	3.6	1.3	10.2 ef
Hydrated	0	+	1.5	1.0	1.5	0	4.0 a
		-	4.2	1.7	4.2	0.1	10.2 ef
	220	+	2.8	1.6	2.1	0.2	6.8 bc
		-	3.8	1.4	4.7	0.1	10.2 ef
	440	+	2.5	0.8	3.0	0.1	6.4 b
		-	3.3	1.2	2.5	0.2	7.2 bc

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

Table 31. Nodulation score for red clover seeded into soil collected from the Demerit site as influenced by phosphorus and inoculation treatments. Each value is the mean of five replications.

Lime treatment	Phosphorus rate	Inoculant	Nodulation Score Color	Position	Number	Size	Total
	kg P_2O_5/ha		units				
Dolomite	0	+	2.2	2.1	3.0	0	7.3 bc*
		-	2.0	1.8	3.2	0	7.0 b
	220	+	3.5	2.0	3.3	0.5	9.3 d
		-	3.0	1.6	4.0	0.7	9.3 d
	440	+	4.5	2.8	4.0	1.0	12.3 f
		-	4.0	2.9	3.8	1.0	11.7 ef
No lime	0	+	2.0	2.5	2.7	0	7.2 bc
		-	1.5	2.2	3.0	0	6.7 b
	220	+	3.2	1.5	4.0	0	8.7 cd
		-	3.0	2.0	3.6	0	8.6 cd
	440	+	4.8	2.3	3.5	0.5	11.1 ef
		-	4.3	3.0	3.0	0.5	10.8 e
Hydrated	0	+	1.0	1.3	2.0	0	4.3 a
		-	0.8	1.7	2.8	0	5.2 a
	220	+	0.4	1.8	2.8	0.2	5.3 a
		-	0.1	2.1	3.0	0	5.2 a
	440	+	0.3	1.7	2.0	0	4.0 a
		-	0.4	1.1	2.3	0.3	4.1 a

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

and hydrated lime soil at 400 kg P_2O_5/ha was 11.0, 10.9, and 4.0, respectively. This interference by hydrated lime in the nodulation of red clover was not reduced by inoculation or applied P. At 0 and 440 kg P_2O_5/ha, the nodulation score for red clover grown with hydrated lime was 4.3 and 4.0, respectively.

For red clover, there was no significant difference in nodulation between the dolomitic and unlimed soils (Table 31). For these plants, P and not inoculation was the most significant influence on nodulation. At 0, 220, and 440 kg P_2O_5/ha, the nodulation score for red clover was 7.8, 9.2, and 10.9, respectively. The applied P increased the percent of pink nodules but had no influence on nodule size, number, or position.

The applied P increased nodule number, color, and size in alfalfa; but for red clover only nodule color was affected. The reason for this differential response to applied P by the legumes is unclear. Possibly the red clover utilizes P more efficiently than alfalfa such that the level of P in the soil at 0 kg P_2O_5/ha was sufficient for maximum nodule number, and the increase in this element caused a higher fixation rate reflected in the nodüle color.

Influence of Aluminum on the Mobility of Phosphurus-32 in Red Clover and Alfalfa

The utilization of P in acidic soil is considered an important factor in the ability of a plant to tolerate low pH. In this study, the mobility of ^{32}P was examined in alfalfa and red clover grown hydroponically in the presence of Al at pH 5.0.

The autoradiographs of the two legumes clearly show that ^{32}P was more mobile in clover than alfalfa (Figures 9 to 11). For both alfalfa and red clover, the roots and nodules are dark, but the leaves of alfalfa are pale compared to the clover. The darker areas indicate the presence of the radioactive phosphorus. Even at 0 ppm Al, the translocation of ^{32}P was more visible in red clover. Foy and Brown (1964) have indicated that tolerance to soil acidity is associated with the utilization of P. White et al. (1976) indicated the presence of polymeric complexes of Al-P in solution, and White (1976) demonstrated the ability of alfalfa to use these complexes as a source of P. Possibly alfalfa can absorb P in these polymeric complexes, but the improved translocation of P in red clover may be accomplished by separating the P from the Al by organic chelation as suggested by Jones (1961). Whatever the mechanism, red clover clearly utilizes P in the presence of Al more efficiently than does alfalfa.

The microanalysis of the nodules by energy dispersive analysis by X-ray (EDAX) indicated differences in Al and P content between alfalfa and red clover (Table 32). The ZAF correction analysis associated with the program used to relate the samples to the standards allows comparisons to be made between biological samples if preparation and handling are the same. All nodules used in this analysis were prepared by the same procedure. The absolute value of the figures, however, must be examined in light of certain artifacts from sample preparation, beam damage, and contamination.

The duplicate readings from the nodule always gave lower values for the two elements. Elemental loss can result from beam damage

Table 32. The levels of Al and P in the nodules of alfalfa and red clover as measured by EDAX. Duplicate readings are presented for each nodule.

Species	Al[1]	Reading	Concentration in nodules Al	P	Al/P
	ppm			%	
Alfalfa	2.5	1	.236	.489	.48
		2	.145	.400	.36
	5.0	1	.228	.456	.50
		2	.198	.410	.48
Red clover	2.5	1	.098	.422	.23
		2	.043	.371	.12
	5.0	1	.063	.403	.16
		2	.027	.300	.09

[1]concentration of Al in the hydroponic solution.

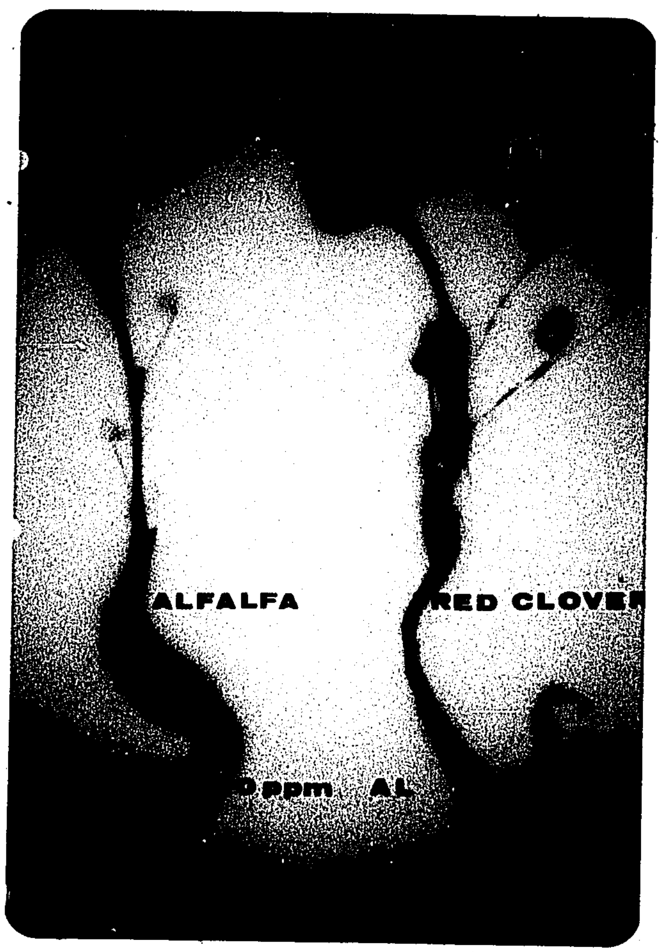


Figure 9. Autoradiograph of alfalfa and red clover grown hydroponically at 0 ppm Al and pH 5.0.

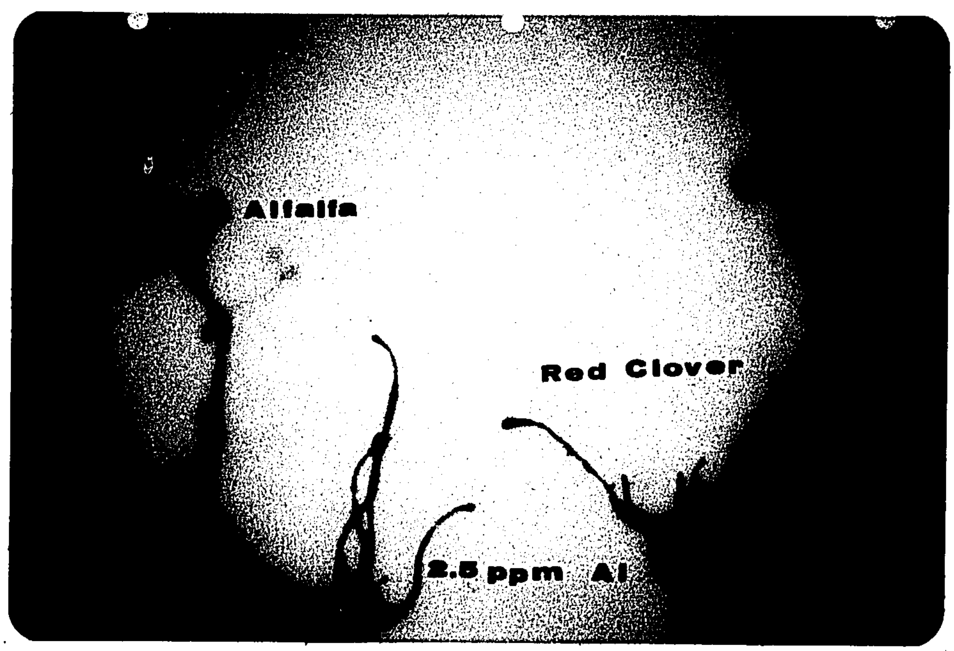


Figure 10. Autoradiograph of alfalfa and red clover grown hydroponically at 2.5 ppm Al and pH 5.0

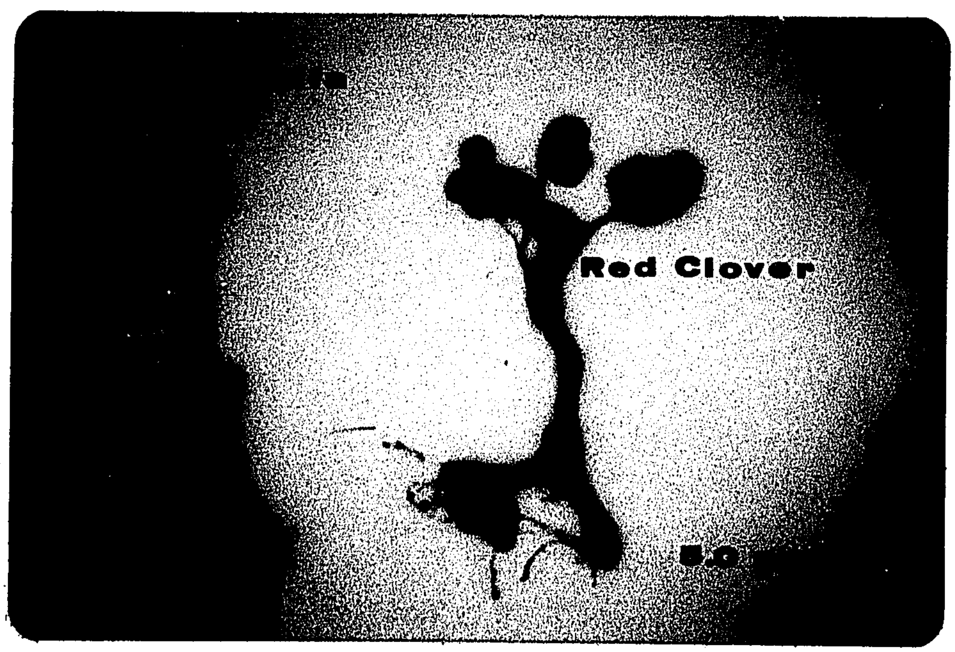


Figure 11. Autoradiograph of alfalfa and red clover grown hydroponically at 5.0 ppm Al and pH 5.0.

and/or contamination of the specimen surface (Echlin, 1978). Hydrocarbon deposits on the wall of the scope have been found to form compounds with the organic breakdown products caused by beam damage of the specimen and these compounds can form substantial films on the surface of the specimen. These coatings can cause a significant reduction in the count rate of soft X-rays (Fiori and Newbury, 1978).

The concentration of Al in the nodules ranged from 0.012 to 0.236 percent which is within the amount found by Sartain and Kamprath (1977) for soybean nodules. It is important to remember that in sample preparation, the cellular water was extracted. In research on animal and plant cells, Panessa-Warren (1979) found that microanalysis of dried cells can result in as much as 10 to 20 percent increase in elemental concentration.

Aluminum levels were significantly higher in alfalfa nodules compared to red clover (Table 32). The Al/P ratio was less in red clover nodules suggesting some mechanism to reduce the translocation of Al to the nodules or an inherent less affinity for the element.

In order to obtain reliable absolute values for the Al in these legume nodules, dependable standards must resemble the samples in chemical and physical properties. Spurr (1975) showed that two standards of different chemical and physical nature gave different readings on the same specimen. Up to this time, the majority of the research has been done with thin sections because of the difficulties in preparing standards for thick or bulky samples.

Laboratory Investigations

Effects of Aluminum and pH on the Growth of Rhizobium meliloti in Culture

Since Al has been shown to be detrimental to the nodulation process in alfalfa, it is possible that the toxicity affects the host plant and the *Rhizobium*. This experiment was conducted to determine the influence of Al and pH on the growth of *R. meliloti* in culture. Three strains of *Rhizobium* were grown in defined media at pH 5.0, 5.5, and 6.0 with 0 and 40 uM Al. Protein levels were monitored over a 24-hour period.

The influence of pH on *R. meliloti* varied between the strains (Figures 12 to 14). Strain F82 showed the most tolerance to acidity (Figure 14) with a generation time at pH 5.0 and 5.5 of four and three hours, respectively. The generation time for strains F70 and F51 at those pH's exceeded six hours.

The influence of Al on protein levels decreased with increasing pH. As indicated in the analysis of variance (Table 33), a significant Al x pH interaction existed for all three strains. At pH 6.0, the Al had no significant influence on the growth of the strains; but at pH 5.0, the element prevented any increase in protein during the 24-hour study period. The tolerance to acidity that the *Rhizobium* showed at pH 5.0 was eliminated by the addition of Al. Keyser and Munns (1979a) found that 48 percent of the cowpea *Rhizobium* strains growing at pH 4.5 and 23 percent of the soybean *Rhizobium* strains growing at pH 4.8 could tolerate added Al.

The recovery of the *Rhizobium* at pH 5.5 from the Al suggests a physiological adaptation of the bacteria to the element or a removal of the Al from the solution over time. Soil bacteria are known to

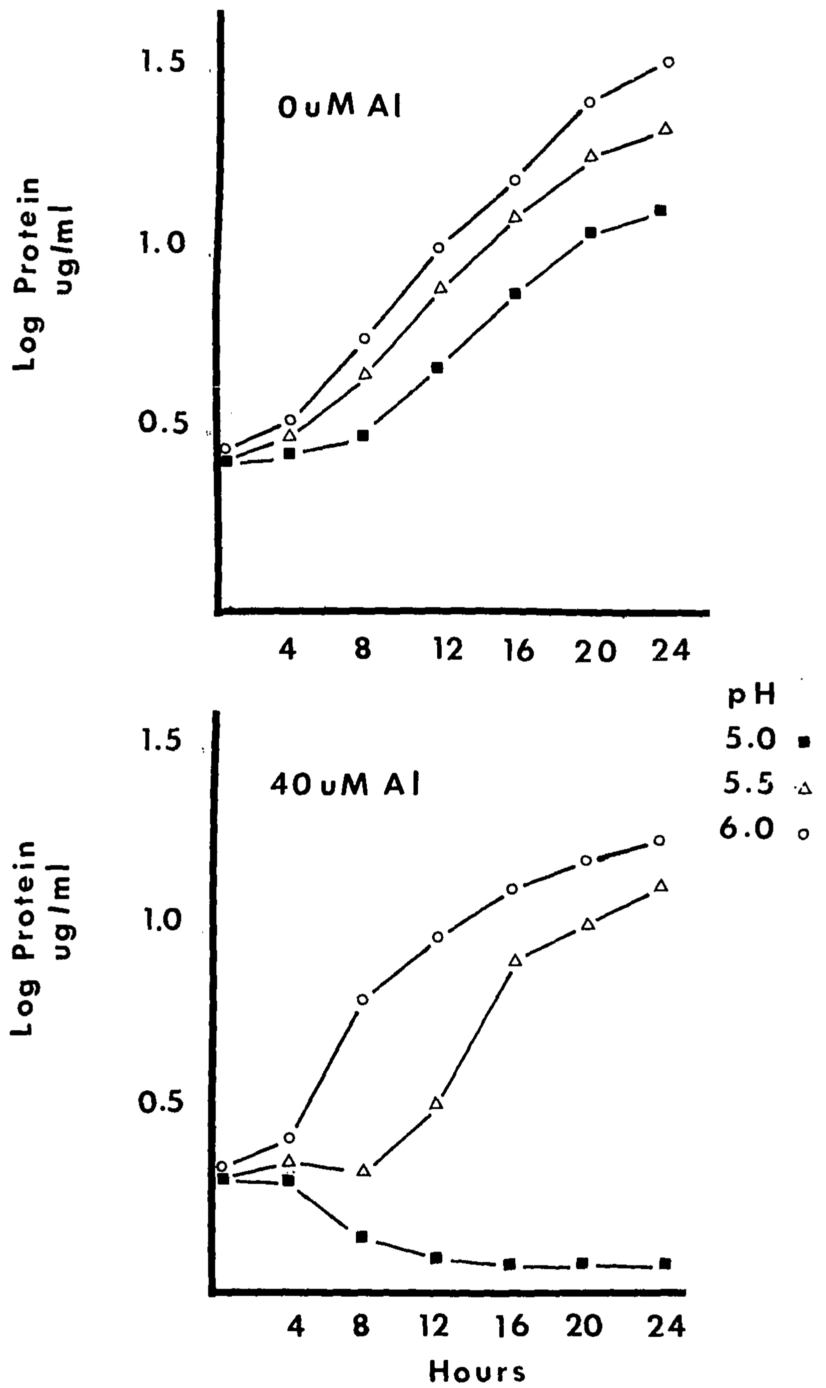


Figure 12. Effect of aluminum and pH on the growth of Rhizobium meliloti strain 102F82.

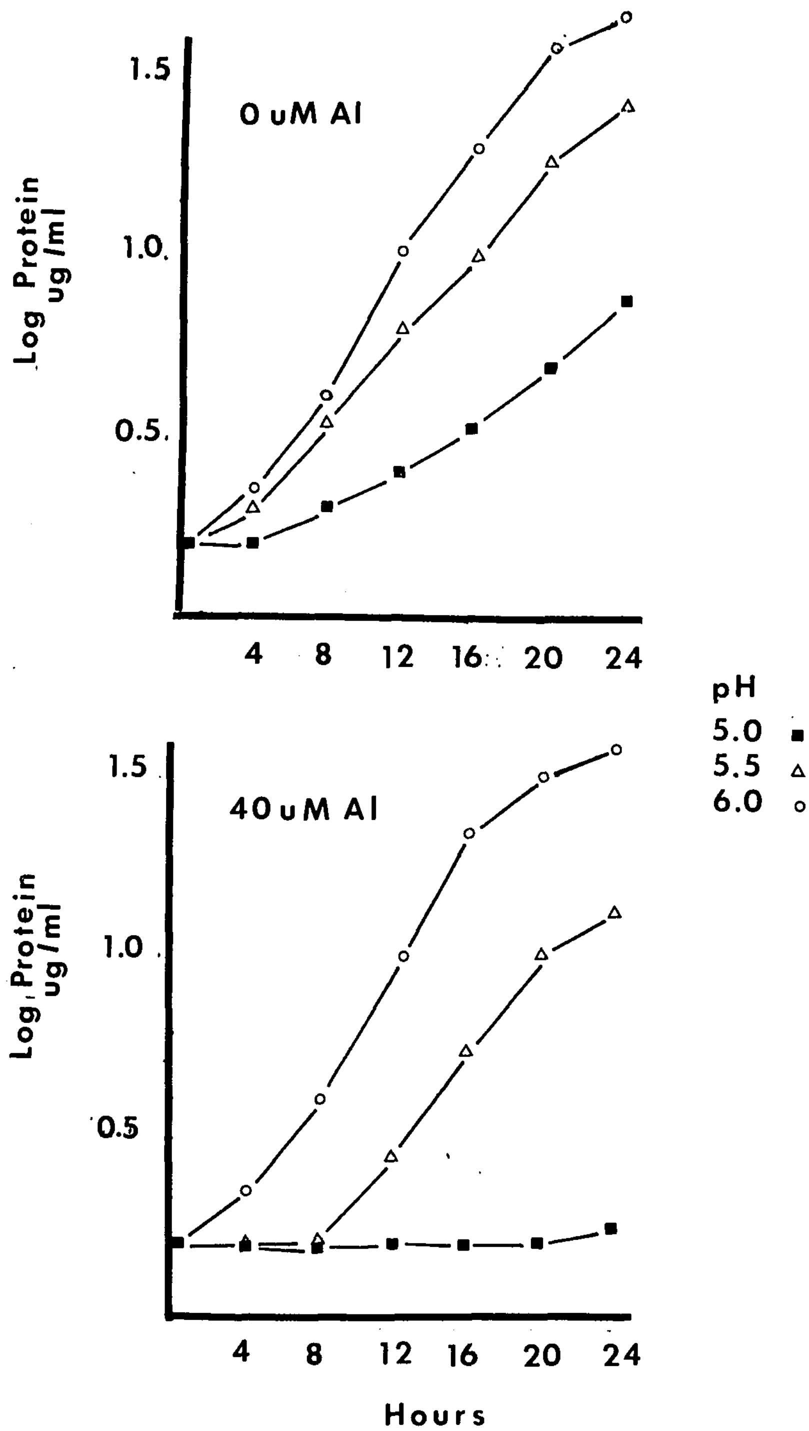


Figure 13. Effect of aluminum and pH on the growth of Rhizobium meliloti strain 102F51.

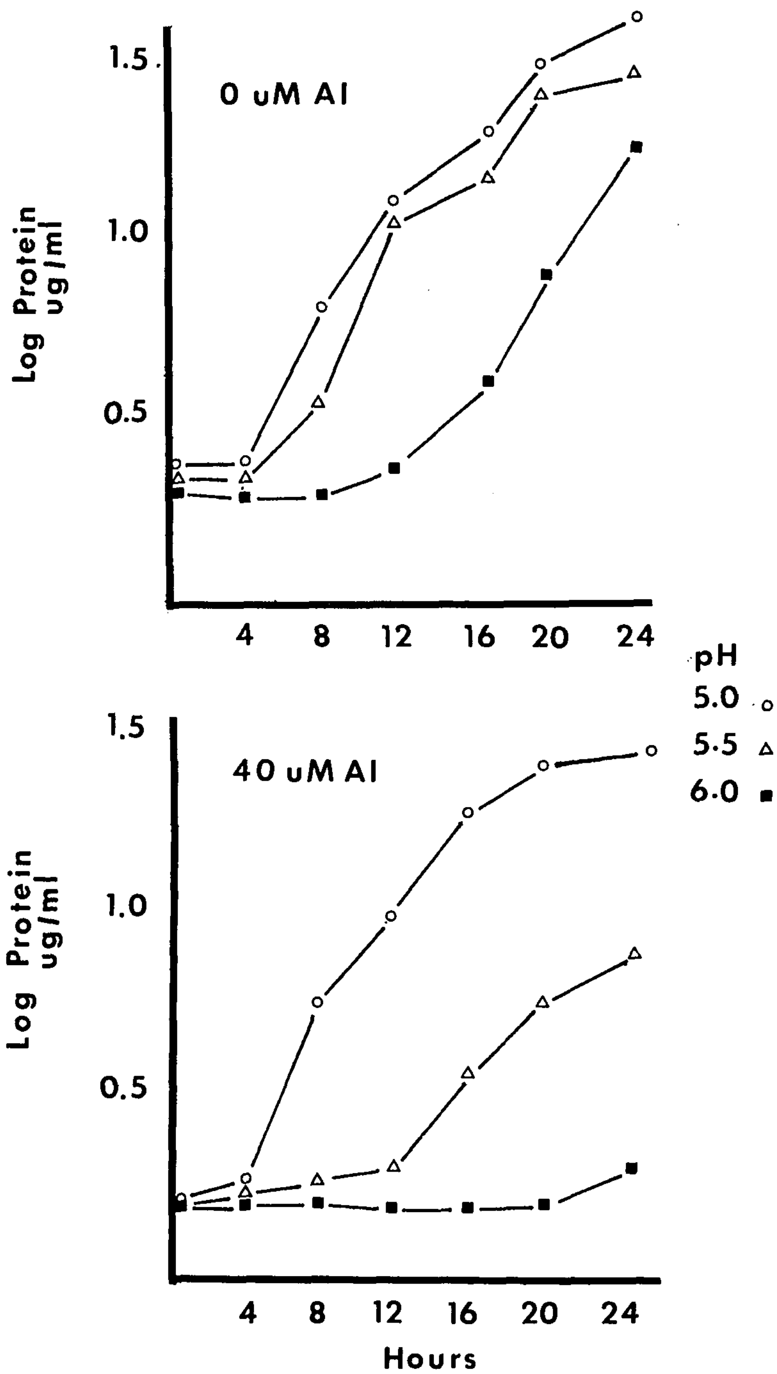


Figure 14. Effect of aluminum and pH on the growth of Rhizobium meliloti strain 102F70.

Table 33. The F ratios from the analysis of variance for the growth of *Rhizobium meliloti* in culture.

Source	Df	Strains F82	F51	F70
Al x pH	2	12.5**	203.1**	9.7**
Al x Time (T)	5	19.0**	101.3**	39.9**
pH x Time	10	19.3**	112.1**	43.2**
Al x pH x T	10	2.2ns	28.2**	2.3ns

** significant at the 0.01 percent level
ns not significant

synthesize a series of organic compounds capable of chelating Al (Silvermann and Munoz, 1970; Duff *et al*., 1963). Possibly under the less stressful conditions of a higher pH, the *R. meliloti* are capable of chelating the Al and reducing its toxicity.

The mechanism of the Al toxicity to the *Rhizobium* may be connected to surface adsorption of the element. Work with soil *Bacillus* in culture with Al indicated that the majority of the element became adsorbed on the walls of the organism (Żwarum *et al*., 1971). Bradley *et al*. (1965) found that when wound bacteria were present in an Al solution most of the element bound to the cell wall within two minutes. It has been shown that adsorbing surfaces can alter bacterial survival and chemical processes (Rubentschik *et al*., 1936). The adsorption of Al to the walls has yet to be shown, but if such phenomenon does occur, the adsorbed Al could interfere in the nutrition of the bacteria and possibly in the nodulation of legumes since the initiation of the process is a surface interaction (Dazzo _et al_., 1976).

Field Investigations

Influence of Lime and Phosphorus on the Growth of Alfalfa and Red Clover in Acidic Soils

Two field experiments were established to study the influence of broadcast/banded lime and P on the growth of alfalfa and red clover in acidic soils. The seedings were done by minimum tillage using a Zip seeder, and the surface vegetation was controlled by an application of glyphosate prior to seeding.

Study 1: Influence of Seeding Date, Banded, and Broadcast Dolomitic Lime on the Growth of Alfalfa and Red Clover. Alfalfa and red clover were sown into a Paxton silt loam on three seeding dates. Dolomitic limestone had been broadcast on all the plots one year prior to the first seeding and banded dolomitic lime was applied with the seed.

The initial soil analysis indicated a pH of 5.0 to a soil depth of 20 cm within the profile and an Al saturation that exceeded 15 percent (Table 34). The broadcast lime moved downward into the top 5 cm of the soil fairly rapidly and by September 1977 the pH in this layer of soil was 5.6 for all three lime rates. At 4.6 and 9.2 t/ha lime, the Al saturation in the top 5 cm of the soil profile was less than 5 percent while the level of Al at 13.8 t/ha lime averaged 1 percent. The level of available P is measured by Bray #1 increased from 7 to 10 ppm in the surface 2 cm of the soil profile. Relating the level of soil P to a qualitative low, medium, or high value is dependent on extractant, soil type, and location. According Baker and Hall (1967) the ranges in Pennsylvania soils was: very high, 80 ppm; high, 50-80 ppm; medium,

Table 34. Initial soil analysis of the Paxton silt loam soil. Each value is the mean of four replications.

Soil depth	pH	Ca	Mg	K	Al	P	Al saturation[1]
cm		Meq/100 g				ppm	%
0 - 2	5.1 a*	1.03 a	0.51 b	0.61 a	0.41 a	7 a	16.01 a
2 - 5	5.0 a	1.51 a	0.43 a	0.51 a	0.40 a	7 a	14.03 a
5 - 10	5.0 a	1.21 a	0.40 a	0.43 a	0.43 a	6 a	17.41 a
10 - 20	4.9 a	1.01 a	0.20 a	0.48 a	0.53 b	5 a	23.87 b

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

[1]Al saturation = Al/(Ca + Mg + K + Al) x 100

30-50 ppm; low, 15-30 ppm; very low, less than 15 ppm. The values established by Olsen and Dean (1969) working with a variety of soils were: high, 20 ppm; medium, 7-20 ppm; low, 3-7 ppm; very low, less than 3 ppm.

By September 1978, 16 months after liming, the pH to a soil depth of 10 cm averaged 5.5 regardless of the lime rate. Aluminum levels were reduced to less than 6 percent saturation down to 10 cm, and the P levels in the surface 2 cm exceeded 10 ppm (Table 35). The soil analysis from the last sampling, September 1979, indicated some change in pH down to 20 cm by the 9.2 and 13.8 t/ha lime rate. Soil Al levels were reduced to less than 2 percent down to 10 cm and available P averaged 14 ppm in the top 2 cm (Table 36).

The soil data shows that the rate of lime application had no significant influence on soil pH in the top 5 cm of the profile (Figure 15). The rate of dolomitic limestone, however, was important in raising pH in deeper horizons with the 13.8 t/ha lime rate being the most effective (Figures 16 and 17). Brown et al. (1956) demonstrated that time rather than lime rate had the major influence on pH change when limestone was applied to a grass sod.

The increase in available P in the surface 5 cm is probably not due to liming but to the P applied at seeding (165 kg P_2O_5/ha). Other research with this Paxton silt loam has shown that P levels do not significantly increase with lime applications (Figure 3 and Table 15). Reeve and Sumner (1970a) have shown that the interaction of soil pH and P varies between soils. Even the reduction of Al does not necessarily indicate any increase in available P. The mechanism of this

Table 35. The September 1978 soil analysis for the Paxton silt loam. Each value is the mean of four replications.

Lime rate	Soil depth	pH	Ca	Mg	K	Al	P	Al saturation
t/ha	cm		Meq/100 g				ppm	%
4.6	0 - 2	6.1 c*	8.1 c	2.0 c	1.0 c	0.01 a	13.1 c	0.09 a
	2 - 5	5.7 b	2.9 b	1.5 b	0.5 b	0.07 b	10.5 b	1.41 b
	5 - 10	5.5 ab	2.0 a	0.7 c	0.3 a	0.19 c	7.0 a	5.92 c
	10 - 20	5.3 a	1.6 a	0.4 a	0.3 a	0.33 a	6.6 a	12.54 d
9.2	0 - 2	6.2 c	8.7 c	2.4 c	1.0 c	0.02 a	12.3 b	0.16 a
	2 - 5	5.9 bc	3.3 b	1.5 b	0.5 b	0.04 a	7.0 a	0.75 b
	5 - 10	5.7 b	2.5 b	0.8 a	0.3 a	0.21 b	7.9 a	5.51 c
	10 - 20	5.3 a	1.0 a	0.6 a	0.2 a	0.22 b	7.0 a	10.89 d
13.8	0 - 2	6.1 c	9.2 c	2.5 c	1.0 c	0.02 a	14.8 b	0.16 a
	2 - 5	5.8 b	4.8 b	2.0 bc	0.8 b	0.05 a	8.8 b	0.65 b
	5 - 10	5.4 a	2.0 a	1.8 ab	0.5 a	0.28 b	6.1 a	6.10 c
	10 - 20	5.3 a	1.4 a	1.3 a	0.5 a	0.37 c	6.6 a	10.30 a

* Within a lime rate, means within a column followed by the same letter are not significantly different at the 0.5 level according to Duncan's New Multiple Range Test.

Table 36. The September 1979 soil analysis for the Paxton silt loam. Each value is the mean of four replications.

Lime rate	Soil depth	pH	Ca	Mg	K	Al	P	Al saturation
t/ha	cm		——	Meq/100 g	——		ppm	%
4.6	0 - 2	6.6 c*	9.8 c	2.9 c	1.0 b	0.01 a	17.1 b	0.07 a
	2 - 5	6.0 b	4.1 b	1.5 b	0.9 b	0.05 a	15.6 b	0.77 b
	5 - 10	5.8 b	2.9 b	0.4 a	0.3 a	0.06 a	8.1 a	1.63 c
	10 - 20	5.4 a	1.4 a	0.7 a	0.3 a	0.20 b	6.5 a	7.72 d
9.2	0 - 2	6.6 c	10.9 c	4.2 c	1.1 c	0.02 a	14.6 b	0.12 a
	2 - 5	6.2 b	6.1 b	2.1 b	0.6 b	0.03 a	10.2 b	0.34 b
	5 - 10	6.0 b	4.3 b	1.8 b	0.3 a	0.11 b	8.0 a	1.68 c
	10 - 20	5.6 a	1.9 a	0.9 a	0.3 a	0.16 b	7.0 a	4.88 d
13.8	0 - 2	6.8 c	12.6 c	3.6 bc	1.0 b	0.02 a	15.1 c	0.12 a
	2 - 5	6.6 c	7.8 b	4.1 c	0.9 b	0.01 a	9.2 b	0.08 a
	5 - 10	6.0 b	6.1 b	2.3 b	0.4 c	0.05 a	7.0 a	0.56 b
	10 - 20	5.7 a	2.0 a	0.7 a	0.4 c	0.16 b	6.5 c	4.91 c

* Within a lime rate, means in a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

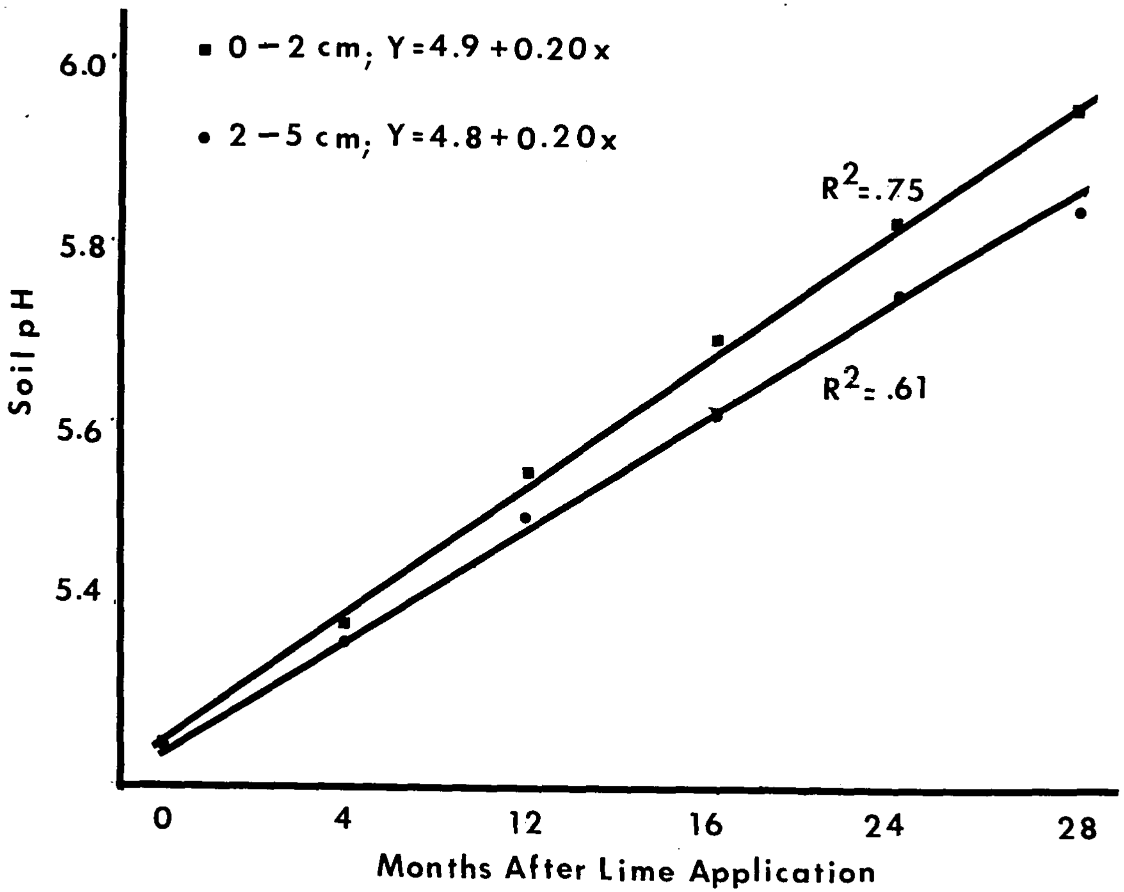


Figure 15. Effect of time on the change in soil pH at 0-2 and 2-5 cm depths in the profile. Lime rates were combined since regression analysis indicated that rate did not influence pH at these depths.

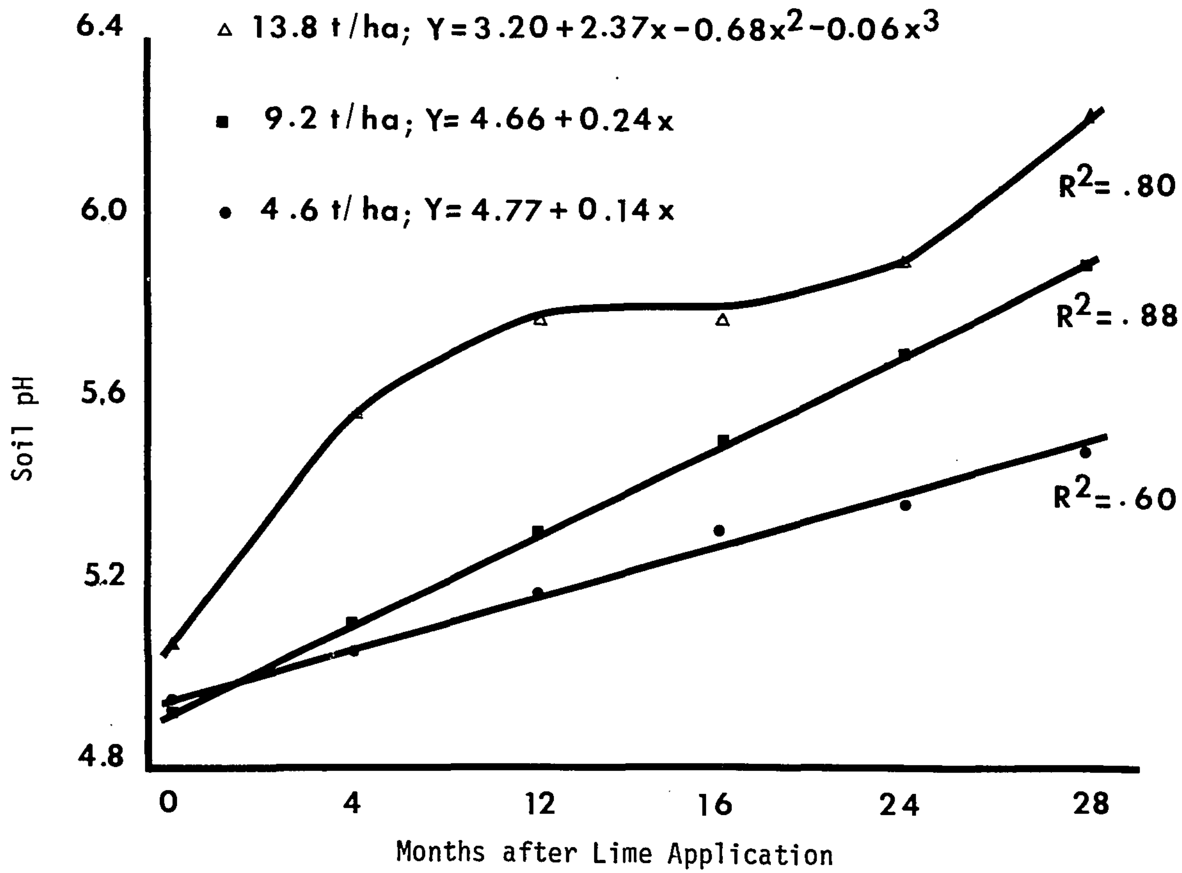


Figure 16. Effect of time and lime rate on the change in soil pH at 5-10 cm depth in the profile as shown by regression analysis.

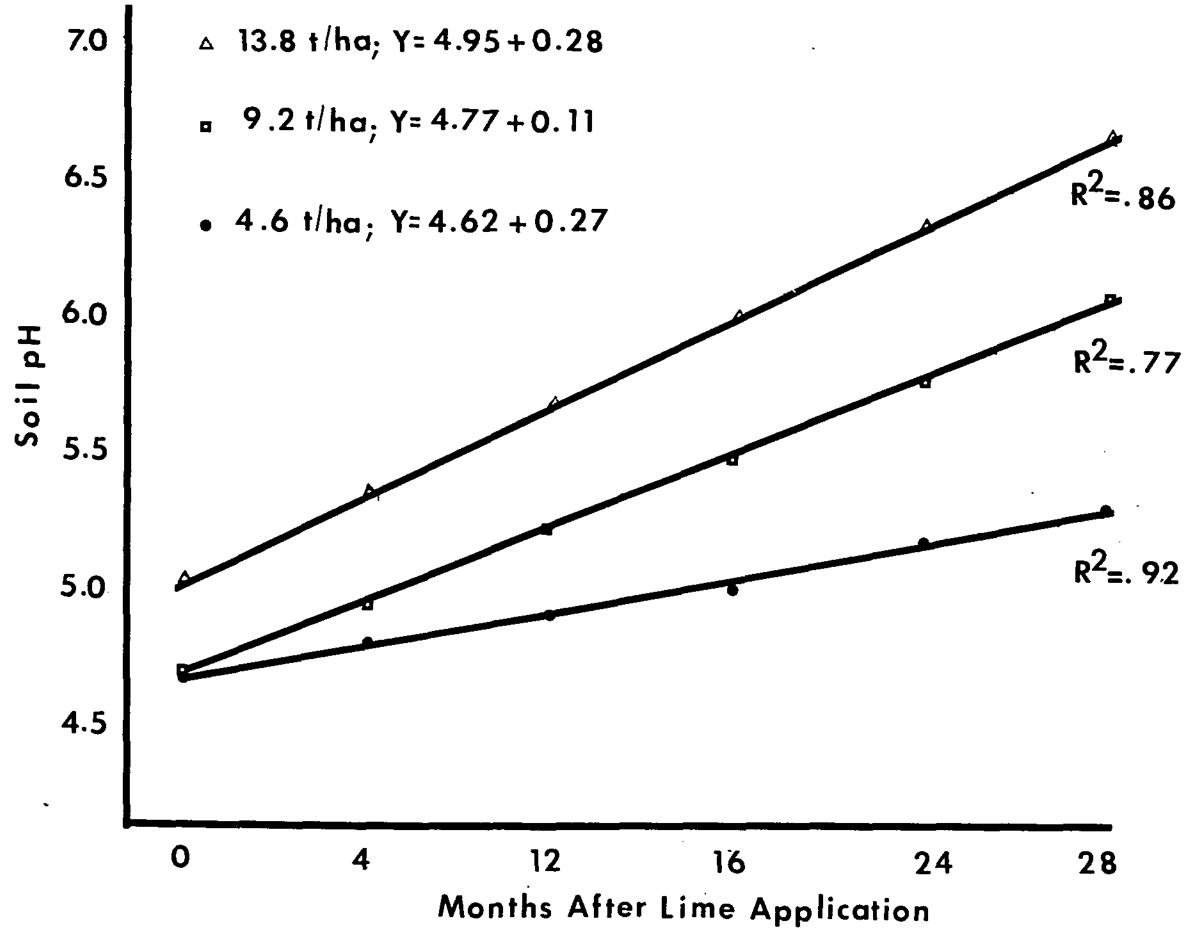


Figure 17. Effect of time and lime rate on the change in soil pH in the 10-20 cm depth in the profile as shown by regression analysis.

P fixation is not entirely understood but is linked to the forms of non-exchangeable Al in the soil (McLean, 1971, 1976).

The one harvest taken in 1978 showed a significant influence of lime rate but not banded lime on alfalfa yield (Table 37). Alfalfa yield at 4.6, 9.2, and 13.8 t/ha lime was 0.36, 0.58, and 0.52 t/ha, respectively. Red clover yields were not influenced by liming or banding and were 20 to 30 percent higher than alfalfa yields. The percent red clover in the plots averaged 89 percent while alfalfa plots averaged 48 percent legume for this harvest.

The tissue composition of the legumes in this 1978 harvest (Table 38) showed a level of Al that exceeded 140 ppm and a P level that averaged 0.22 percent in alfalfa. For red clover, Al levels were less than 70 ppm and P levels were similar to those in alfalfa. No significant correlation existed between change in soil pH and levels of plant Al or P for either legume. It must be remembered, however, that P levels in plant tissue do not necessarily indicate the availability of P to metabolism due to the possibility of Al-P interactions within the plant. Clarkson (1965) showed that a large portion of the P in barley roots of plants grown at high Al levels hydroponically made no contribution to the P incorporated into intermediates in metabolism. The Al/P ratio in alfalfa and red clover foliage was 0.068 and 0.029, respectively, which might indicate a difference in terms of P translocation in the two legumes.

The first harvest in 1979 showed a significant interaction between seeding date and lime for alfalfa (Table 39) and for red clover (Table 40). Alfalfa yields at 4.6, 9.2, and 13.8 t/ha lime for the May 1978 seeding date were 4.4, 4.0, and 3.7 t/ha, respectively;

Table 37. Yields of red clover and alfalfa from the August 1978 harvest. Each value is the mean of four replications.

Seeding date	Lime rate	Banding	Species Alfalfa	Species Red clover
	t/ha		t/ha	
May 1978	4.6	+	0.39 a*	0.61 a
		-	0.33 a	0.65 a
	9.2	+	0.58 b	0.63 a
		-	0.59 b	0.59 a
	13.8	+	0.54 b	0.71 a
		-	0.50 b	0.71 a

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

Table 38. Tissue composition of alfalfa and red clover from the 1978 harvest of the Harmony Hill Farm. Each value is the mean of four replications. The values for banded and non-banded legume were combined; analysis of variance indicated that banding had no significant influence on tissue composition.

Species	Lime rate	N	Ca	Mg	P	K	Al
	t/ha	%					ppm
Alfalfa	4.6	3.23	0.93	0.32	0.24	2.5	156
	9.2	3.22	1.04	0.34	0.22	2.5	147
	13.8	3.32	0.94	0.34	0.23	2.5	149
Red clover	4.6	3.41	1.23	0.43	0.23	2.9	70
	9.2	3.58	1.18	0.43	0.24	2.5	61
	13.8	3.42	1.16	0.42	0.25	3.0	66

Table 39. Yield of alfalfa in 1979 from the Harmony Hill Farm. Each value is the mean of four replications.

Seeding date	Lime rate	Banding	Harvests June	July	October	Total
	t/ha			t/ha		
May 1978	4.6	-	3.9 a*	3.0 a	1.7 a	8.6 a
		+	4.4 a	3.5 a	1.2 a	9.0 a
	9.2	-	3.8 a	2.7 a	1.4 a	7.9 a
		+	4.0 a	3.0 a	1.0 a	8.0 a
	13.8	-	4.1 a	3.2 a	1.2 a	8.5 a
		+	3.7 a	3.2 a	0.9 a	7.8 a
August 1978	4.6	-	0.3 a	0.6 a	0.7 a	1.6 a
		+	0.6 a	0.8 a	0.8 a	2.2 ab
	9.2	-	0.7 ab	1.4 b	0.9 a	3.0 b
		+	0.7 ab	1.4 b	0.9 a	3.0 b
	13.8	-	1.0 b	0.7 a	0.9 a	2.6 ab
		+	1.0 b	0.6 a	0.6 a	2.2 ab
May 1979	4.6	-	-[1]	0.7 a	0.9 a	1.6 a
		+	-	1.0 ab	0.6 a	1.6 a
	9.2	-	-	1.0 ab	0.9 a	1.9 ab
		+	-	0.7 a	0.9 a	1.6 a
	13.8	-	-	1.5 b	0.9 a	2.4 b
		+	-	0.9 ab	1.0 a	1.9 ab

*Within a seeding date, means in a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.
[1]This seeding was not harvested in June.

Table 40. Yield of red clover in 1979 from Harmony Hill Farm. Each value is the mean of four replications.

Seeding date	Lime rate	Banding	Harvests: June	July	October	Total
	t/ha			t/ha		
May 1978	4.6	-	2.5 a*	1.5 a	0.2 a	4.2 a
		+	2.5 a	1.5 a	0.3 a	4.3 a
	9.2	-	2.4 a	2.0 a	0.3 a	4.7 a
		+	2.4 a	2.0 a	0.4 a	4.8 a
	13.8	-	2.5 a	1.6 a	0.5 a	4.6 a
		+	2.1 a	1.9 a	0.4 a	4.4 a
August 1978	4.6	-	0.7 a	1.0 a	0.6 ab	2.3 a
		+	0.7 a	1.0 a	0.5 a	2.5 a
	9.2	-	0.4 a	1.4 a	0.9 b	2.7 ab
		+	0.4 a	1.4 a	0.9 b	2.7 ab
	13.8	-	1.4 b	0.9 a	0.8 ab	3.1 b
		+	1.4 b	0.9 a	0.6 ab	3.0 b
May 1979	4.6	-	-[1]	0.4 a	0.7 a	1.1 a
		+	-	0.3 a	0.8 a	1.1 a
	9.2	-	-	0.9 ab	0.9 a	1.8 ab
		+	-	0.9 ab	0.9 a	1.8 ab
	13.8	-	-	1.4 b	0.9 a	2.3 b
		+	-	1.3 b	0.9 a	2.2 b

*Within a seeding date, means in a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

[1]This seeding was not harvested in June.

and for August 1978 seeding dates the yields were 0.6, 0.7, and 1.0 t/ha, respectively. The yields for this August seeding for both legumes was low which was probably due to competition from grasses and low rainfall in the weeks following seeding (Table 41). From the seedings made in May and August 1978, yields for red clover were appreciably lower than those of alfalfa. The percent red clover in the plots decreased from 89 percent in 1978 to 45 percent in 1979.

Analysis of the red clover roots indicated necrosis in the pith of the crown and inspection of the tissue showed the presence of a severe infection of Fusarium solani which is commonly associated with root rot (Taylor, 1975). According to Cressman (1967) internal breakdown in red clover is not caused by any pathogen but is a physiological disorder which can be aggravated by poor nutrition or environmental stress. Siddiqui et al. (1968) found a direct relationship between root rot severity, the number of Fusarium per root and clipping frequency. Cressman (1967) states that the survival of red clover is dependent on the capacity of the plants to produce a secondary root system before the original crown deteriorates. Clipping, in effect, removes photosynthetic capacity and probably reduces root reserves which would weaken the plants' ability to develop a secondary root system.

In August 1979, all three seeding dates were harvested for alfalfa (Table 39) and red clover (Table 40). The yields of both legumes were significantly influenced by seeding date. The yields of alfalfa for the May 1978, August 1978, and May 1979 seeding dates averaged across the lime rates were 3.1, 0.9, and 1.0 t/ha, respectively. For red clover, the highest yields were found the May

Table 41. Rainfall from May to September on a weekly basis for 1977, 1978, and 1979. The rainfall data presented here were recorded at the official Durham, New Hampshire, Weather Station.

Week ending		Years	
	1977	1978	1979
	cm		
May 7	0.15	0	1.40[3]
14	5.55	1.37	1.02
21	0.25	11.20[1]	1.47
28	0	0.69	7.82
June 4	1.85	3.78	1.32
11	4.00	2.79	0.30
18	0.35	0.46	1.30
25	3.10	0.76	0.99
July 2	2.65	0.69	1.68
9	0.55	0.10	0
16	0.40	1.37	0
23	0.10	0.15	2.18
30	3.05	0.89	0.90
August 6	2.30	3.18[2]	2.26
13	0.75	0.63	4.85
20	0.15	0	1.22
27	2.95	1.42	0.18
Total	28.15	29.48	28.89

Week that seeding was made:

[1] May 25, 1978

[2] August 11, 1978

[3] May 7, 1979

1979 seeding while for alfalfa, the May 1978 seeding gave the best results.

In the third harvest of 1979, taken in late October, alfalfa yield was not significantly influenced by seeding date, broadcast, or banded lime (Table 39). The average yield for alfalfa in this harvest was 1.0 t/ha. The yield of red clover for May 1978, August 1978, and May 1979 seeding dates averaged across the lime rates was 0.4, 0.7, and 0.9 t/ha, respectively (Table 40).

Lime rate and seeding date did not have any significant influence on alfalfa nodule number or weight (Table 42). Date (1970) indicated that the nodulation pattern of mature alfalfa is generally represented by a few large lobed nodules. The average nodule weight for alfalfa was 4.6 mg. Nodule number in red clover did increase significantly with seeding date (Table 43). Red clover has been shown to have numerous small nodules spread over its roots (Date, 1970). For the May 1978 seeding date, the total nodule weight for red clover averaged 20.9 mg for 43 nodules;and for alfalfa, the weight averaged 23.8 mg for 6 nodules.

The failure of banding lime to influence yield was probably connected to the reduced acidity in the soil profile induced by the broadcast lime. By the first seeding, May 1978, the soil pH in the top 5 cm of the soil profile averaged 5.8, and the Al saturation had been reduced to less than 1 percent at that depth. The level of Ca in the soil exceeded 1500 ppm and the level of available P averaged 10 ppm. The broadcast lime penetrated the soil profile enough to provide an adequate environment for seedling growth and nodulation.

Table 42. Nodule number and weight for alfalfa as influenced by limestone treatment. Each value is the mean of four replications.

Seeding date	Lime Rate	Nodule number per nodule	Weight per nodule
	t/ha		mg
May 1978	4.6	5.7[1]	4.0
	13.8	6.3	3.6
August 1978	4.6	4.3	4.7
	13.8	5.4	4.2
May 1979	4.6	5.9	4.2
	13.8	5.5	5.0

[1]No significant differences in alfalfa nodule number or weight due to seeding date and/or lime rate according to analysis of variance.

Table 43. Nodule number and weight for red clover as influenced by limestone treatment. Each value is the mean of four replications.

Seeding date	Lime rate	Nodule number per nodule	Weight per nodule
	t/ha		mg
May 1978	4.6	39 c*	0.6 a
	13.8	46 c	0.4 a
August 1978	4.6	24 b	0.9 a
	13.8	19 ab	0.8 a
May 1979	4.6	13 a	0.8 a
	13.8	12 a	1.1 a

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

The data in the experiment indicated that good yields of alfalfa can be achieved in the year following establishment by reducing acidity in the top 10 cm of the soil profile if adequate P levels exist. Longevity of the stand has not yet been determined under such conditions. In this experiment, the levels of Al were reduced from an average of 15 percent to 1 percent, and the available P levels increased from 7 to 14 ppm in the top 5 cm of the soil following limestone and P application. While the effects of subsoil acidity on long-term alfalfa growth are not known, the continuation of this experiment should provide useful information on this problem.

<u>Study 2: Influence of Broadcast Limestone and Broadcast and Banded Phosphurous on the Growth of Alfalfa and Red Clover</u>. Alfalfa and red clover were established at the Demerit Livestock Farm in Lee, New Hampshire, in a Hinckley sandy loam with a Zip seeder in August 1978. Dolomitic lime, hydrated lime, and a lime check treatment were established in June 1978. Broadcast and banded P were applied in August at seeding. Three harvests were taken in 1979 for both legumes.

The initial analysis of the soil taken in August 1977 showed levels of Ca, Mg, and K in the top 5 cm of the soil profile of 520, 180, and 70 ppm, respectively (Table 44). Soil pH was a uniform 5.6 down to 20 cm, but Al saturation increased from 2.7 percent at the 0-2 cm depth to 11.6 percent at 10-20 cm. The levels of available P were in the medium range according to the guidelines established by Olsen and Dean (1969).

The changes in soil chemistry due to hydrated lime were obvious in July 1978, one month after lime application (Table 45). Soil pH averaged over the top 5 cm of the profile at 1.2 and 2.3 t/ha hydrated

Table 44. Initial chemical analysis of the Hinckley sandy loam soil. Each value is the mean of four replications.

Soil depth	pH	Ca	Mg	K	Al	P	Al saturation[1]
cm		Meq/100 g				ppm	%
0 - 2	5.6 a*	3.2 c	2.0 b	1.0 a	0.17 a	15 b	2.7 a
2 - 5	5.6 a	2.0 b	1.0 ab	1.0 a	0.20 ab	16 b	6.3 ab
5 - 10	5.6 a	1.1 ab	0.6 a	0.9 a	0.27 bc	10 a	9.1 bc
10 - 20	5.6 a	0.9 a	0.6 a	0.7 a	0.29 c	9 a	11.6 c

[1]Al saturation = Al/(Ca + Mg + K + Al) x 100

*Means within a column followed by the same letter are not significantly different at 0.05 percent level according to Duncan's New Multiple Range Test.

Table 45. Soil analysis of the Hinckley sandy loam in July 1978. Each value is the mean of four replications.

Lime treatments	Soil depth	pH	Ca	Mg	K	Al	P	Al saturation[1]
	cm		Meq/100 g				ppm	%
No lime	0 - 2	5.6 a*	3.8 c	2.4 c	1.6 b	0.16 a	15 a	2.0 a
	2 - 5	5.5 a	2.0 b	1.2 b	1.0 a	0.18 a	18 b	4.1 a
	5 - 10	5.6 a	1.1 a	0.7 a	0.6 a	0.33 b	10 a	11.8 b
Hydrated 1.12 t/ha	0 - 2	6.5 b	9.0 c	2.7 c	1.8 b	0 a	12 a	0 a
	2 - 5	6.2 b	3.5 b	1.5 b	0.8 a	0 a	10 a	0 a
	5 - 10	5.8 a	1.6 a	0.6 a	0.8 a	0.15 b	10 a	4.8 b
2.3 t/ha	0 - 2	6.8 b	12.6 c	2.2 c	1.1 a	0 a	11 a	0 a
	2 - 5	6.7 b	3.6 b	1.4 b	1.0 a	0 a	11 a	0 a
	5 - 10	6.0 a	1.4 a	0.2 a	0.7 a	0.10 b	10 a	4.2 b
Dolomitic 6.90 t/ha	0 - 2	5.8 b	8.9 c	4.7 b	1.1 a	0.01 a	15 b	0.1 a
	2 - 5	5.7 ab	3.5 b	1.0 a	1.0 a	0.11 b	16 b	2.0 b
	5 - 10	5.6 a	1.9 a	1.0 a	0.8 a	0.27 c	10 a	7.2 c
13.8 t/ha	0 - 2	5.9 c	8.6 b	4.6 c	1.6 b	0.11 a	16 b	0.7 a
	2 - 5	5.7 b	2.3 a	1.6 b	1.0 a	0.13 a	15 b	2.6 b
	5 - 10	5.5 a	2.8 a	0.8 a	0.8 a	0.19 b	10 a	4.1 c

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

[1]Al saturation = Al/(Ca + Mg + K + Al) x 100

lime was 6.3 and 6.5, respectively. Aluminum saturation was reduced to 0 in the 0-2 and 2-5 cm depths and to 4 percent in the 5-10 cm depth. There were no significant changes in Mg or K in the soil profile, but Ca levels exceeded 1800 ppm in the 0-2 cm depth and 700 in the 2-5 cm depth. Soil P levels significantly decreased from 16 ppm in the top 5 cm in August 1977 to 10 ppm in July 1978. The last soil sample which was collected in September 1979 showed no significant changes in pH, Al, Ca, K, or P from the July 1978 sample in the plots with 0 kg P_2O_5/ha (Table 45).

In the plots that received dolomitic limestone, changes in soil chemistry continued throughout the experiment. Soil pH averaged over the top 5 cm of the profile in July 1978 and September 1979 was 5.8 and 6.4 at 13.8 t/ha dolomitic lime. Aluminum saturation at this high lime rate of dolomite was 0.7, 2.6, and 4.1 percent in the 0-2, 2-5, and 5-10 cm soil depth, respectively, in July 1978. By September 1979, the Al saturation was reduced to less than 2 percent down to 10 cm at both rates of dolomitic limestone (Table 48).

The application of broadcast P reduced the Al levels in the unlimed plots to levels comparable to those in the hydrated and dolomitic plots in the top 5 cm of the soil profile (Figure 18) and increased the availability of soil P in all the plots (Tables 46, 47, 48). The increase in available P was smaller in the hydrated plots compared to the unlimed and dolomitic lime plots (Table 46, 47, 48); the hydrated lime seem to reduce the amount of P extractable by Bray #1.

The single application of 240 kg banded P_2O_5/ha produced significantly higher yields of alfalfa than the single application of 400 kg broadcast P_2O_5/ha in the unlimed and hydrated plots. The

Table 46. Soil analysis of the unlimed Hinckley sandy loam in September 1979. Each value is the mean of four replications.

Phosphorus rates	Soil depth	pH	Ca	Mg	K	Al	P	Al saturation[1]
kg P_2O_5/ha	cm		Meq/100 g				ppm	%
0	0 - 2	5.6 a*	3.4 e	2.0 ef	1.5 e	0.15 b	18 b	2.1 c
	2 - 5	5.7 a	1.5 b	1.0 cd	1.0 c	0.15 b	16 b	4.1 d
	5 - 10	5.6 a	1.0 a	0.3 a	0.8 b	0.13 b	10 a	9.3 f
	10 - 20	5.6 a	1.0 a	0.3 a	0.3 a	0.19 c	10 a	9.1 f
220	0 - 2	5.7 a	3.0 e	2.3 fg	1.0 cd	0 a	70 e	0 a
	2 - 5	5.6 a	2.0 c	1.2 d	1.0 cd	0.02 a	48 c	0.5 ab
	5 - 10	5.6 a	0.9 a	0.6 ab	0.9 bc	0.07 a	20 b	2.8 c
	10 - 20	5.6 a	0.8 a	0.7 bc	0.6 ab	0.22 c	19 b	9.5 f
440	0 - 2	5.7 a	2.7 d	2.5 g	1.3 d	0.02 a	128 f	0.3 a
	2 - 5	5.6 a	2.0 d	1.7 e	1.0 cd	0.03 a	61 d	0.6 b
	5 - 10	5.6 a	0.8 a	1.2 d	0.8 bc	0.12 b	58 d	4.1 d
	10 - 20	5.6 a	1.0 a	0.5 ab	0.9 bc	0.21 c	18 b	8.0 e

[1]Al saturation = Al/(Ca + Mg + Al) x 100

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

Table 47. Soil analysis of the Hinckley sandy loam treated with 2.3 t/ha hydrated lime and sampled in September 1979. Each value is the mean of four replications.

Phosphorus rate	Soil depth	pH	Ca	Mg	K	Al	P	Al saturation[1]
kg P_2O_5/ha	cm		Meq/100 g				ppm	%
0	0 - 2	6.1 e*	10.0 d	1.2 bc	1.0 a	0.01a	19 b	0.07 a
	2 - 5	5.9 cd	4.8 bc	1.0 b	1.0 a	0.11 b	18 b	0.60 b
	5 - 10	5.6 a	2.0 a	1.0 b	0.8 a	0.13 b	13 a	3.30 d
	10 - 20	5.6 a	1.0 a	0.5 a	0.8 a	0.32 d	12 a	12.20 g
220	0 - 2	6.2 f	10.0 d	1.4 c	0.9 a	0 a	70 e	0 a
	2 - 5	6.4 g	4.0 bc	1.0 b	1.0 a	0.03 a	64 d	0.50 b
	5 - 10	6.0 de	2.0 ab	0.9 b	0.8 a	0.04 a	46 c	1.10 c
	10 - 20	5.8 bc	1.0 a	0.8 b	0.8 a	0.23 c	18 f	8.23 e
440	0 - 2	6.4 g	10.2 d	1.0 b	0.8 a	0 a	88 f	0 a
	2 - 5	6.1 ef	5.5 c	1.0 b	0.8 a	0 a	64 d	0 a
	5 - 10	5.8 b	3.0 ab	0.9 b	1.0 a	0.20 a	63 d	3.9 d
	10 - 20	5.7 ab	1.0 a	0.8 b	1.0 a	0.30 a	20 b	9.7 f

[1]Al saturation = Al/(Ca + Mg + K) x 100

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

Table 48. Soil analysis of the Hinckley sandy loam treated with 13.8 t/ha dolomitic limestone and sampled in September 1979. Each value is the mean of four replications.

Phosphorus rate	Soil depth	pH	Ca	Mg	K	Al	P	Al saturation[1]
kg P_2O_5/ha	cm		——— Meq/100 g ———				ppm	%
0	0 - 2	6.2 de*	10.1 d	5.2 f	1.2 ab	0 a	17 b	0 a
	2 - 5	6.3 ef	7.8 c	3.2 d	1.0 a	0.02 a	18 b	0.17 a
	5 - 10	5.7 bc	1.2 a	2.1 b	0.9 a	0.08 ab	10 a	1.10 b
	10 - 20	5.8 d	1.0 a	0.5 a	0.8 a	0.28 d	10 a	10.85 c
220	0 - 2	6.4 f	11. 2 e	4.1 e	1.5 b	0 a	95 f	0 a
	2 - 5	6.2 de	8.1 c	3.0 cd	1.0 a	0.04 a	64 d	0.30 a
	5 - 10	5.8 c	3.7 b	2.0 b	0.8 a	0.08 ab	47 c	1.20 b
	10 - 20	5.6 b	1.0 a	0.5 a	0.8 a	0.21 c	10 a	9.36 c
440	0 - 2	6.6 g	9.6 d	2.3 b	1.0 a	0 a	135 g	0 a
	2 - 5	6.1 d	7.5 c	2.8 c	1.0 a	0.02 a	76 e	0.20 a
	5 - 10	5.7 cd	3.2 b	2.1 b	0.8 a	0.11 b	40 c	1.80 b
	10 - 20	5.4 a	1.0 a	0.5 a	0.8 a	0.30 d	15 a	9.31 c

[1]Al saturation = Al/(Ca + Mg + K + Al) x 100

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

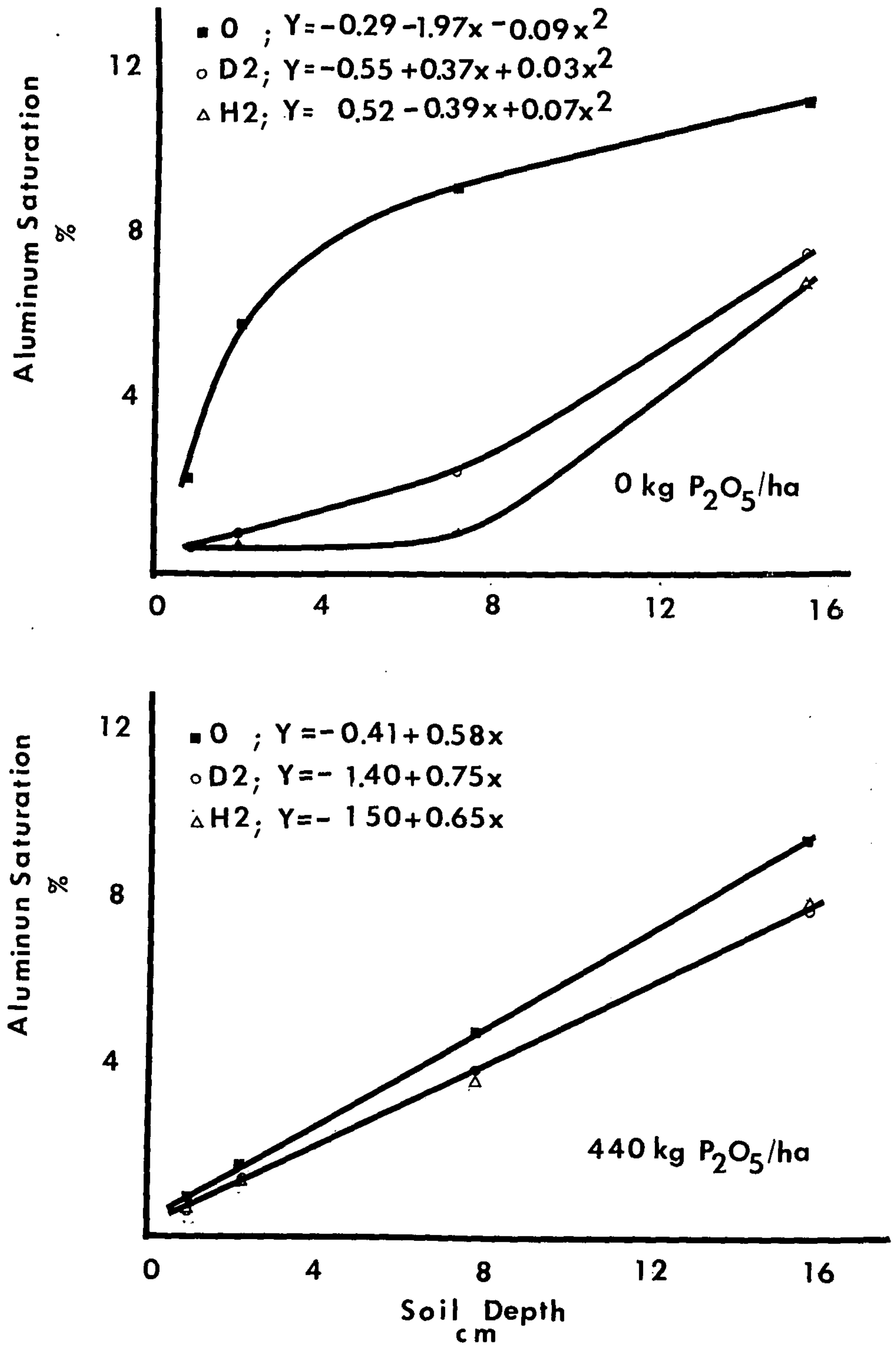


Figure 18. Effect of phosphorus fertilizer on aluminum saturation at various soil depths as shown by regression analysis.

combination of these two P treatments increased the yields significantly over the other P treatments at 13.8 t/ha dolomitic lime (Table 49). The yields of alfalfa with the single banded treatment in the unlimed, 2.3 t/ha hydrated lime and 13.8 t/ha dolomitic lime plots were 6.6, 6.7, and 6.5 t/ha, respectively. The addition of the banded P eliminated the lime response in alfalfa.

With red clover, the single application of banded P was more effective than broadcast P in increasing yields only in the unlimed plots (Table 50). The yields of red clover with this single banded P treatment in the unlimed, 2.3 t/ha hydrated and 13.8 t/ha dolomitic lime plots were 6.4, 3.6, and 5.4 t/ha, respectively. The hydrated lime significantly reduced clover yields relative to the dolomitic lime and unlimed plots.

The influence of banded P on forage legume growth has been discussed by several researchers (Brown, 1950; Levessue and Ketcheson, 1963; Taylor, 1975). According to Levessue and Ketcheson banded P is beneficial to alfalfa seedling growth in the first four to six weeks after seeding but offers no yield advantage. Brown (1950) stated that banded superphosphate increased seedling growth for alfalfa, but the yields did not differ significantly from plants treated with broadcast superphosphate. The increase in yield of the two legumes from the banded P treatments was probably due to the N added from the mono-ammonium phosphate (MAP). The MAP is liquid and was used due to its solubility and usefulness as a treatment. This compound is 12 percent N which means that 27.5 kg N/ha was added at the high rate of banding. According to Rhykerd and Overdahl (1972), the banding of P and N is

Table 49. Total yield for alfalfa in 1979 from the Demerit experiment. Each value is the mean of four replications.

Lime Treatments	Phosphorus Treatments kg P_2O_5/ha						
	Bnd[1] 240	Brd[2] 440	Bnd 120 Brd 220	Bnd 120 Brd 440	Bnd 240 Brd 220	Bnd 240 Brd 440	$\bar{x}$
	t/ha						
No lime	6.6 b*C**	4.3 aA	6.3 aBC	5.9 bB	4.7 aA	6.8 bC	5.8
1.12 t/ha Hydrated	5.8 aBC	5.3 bA	6.2 aC	5.5 bAB	5.1 abA	5.8 aBC	5.6
2.30 t/ha Hydrated	6.7 bC	5.8 bcB	7.0 bC	4.9 aA	5.7 bB	6.9 bC	6.2
6.90 t/ha Dolomitic	6.0 abAB	6.3 cB	6.9 bC	5.8 bA	6.1 cAB	6.4 bB	6.2
13.8 t/ha Dolomitic	6.5 bAB	6.3 cA	7.2 bC	6.7 cB	6.3 cA	7.5 cC	6.8

[1]banded [2]broadcast

*Means within a column followed by the same small letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

**Means within a row followed by the same capital letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

Table 50. Total yield of red clover in 1979 from the Demerit experiment. Each value is the mean of three replications.

Lime Treatments	Bnd[1] 240	Brd[2] 440	Bnd 120 Brd 220	Bnd 120 Brd 440	Bnd 240 Brd 220	Bnd 240 Brd 440	$\bar{x}$
	Phosphorus Treatments kg P_2O_5/ha						
	t/ha						
No lime	6.4 d*B**	5.4 bcA	4.7 aA	6.7 cB	6.2 bB	5.3 bA	5.8
1.12 t/ha Hydrated	5.0 bcC	5.4 cD	4.3 aAB	4.8 aBC	4.1 aA	6.0 cD	4.9
2.30 t/ha Hydrated	3.6 aA	3.5 aA	4.7 aC	4.5 aBC	3.9 aAB	4.0 aAB	4.0
6.9 t/ha Dolomitic	4.6 bA	4.9 bA	6.1 bC	5.6 bB	6.0 bC	6.1 cC	5.7
13.8 t/ha Dolomitic	5.4 cA	5.8 cAB	7.4 cD	6.0 bCD	6.5 bC	7.8 dD	6.1

[1]banded [2]broadcast

*Means within a column followed by the same small letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

**Means within a row followed by the same capital letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

more effective in improving alfalfa growth than banded P alone provided that the rates of N are kept low.

There were no significant correlations between levels of Al and P in alfalfa tissue with lime, P application or soil characteristics (Table 51). The level of tissue Al in the unlimed, 2.3 t/ha hydrated and 13.8 t/ha dolomitic lime plots was 99, 87, and 89 ppm, respectively. The level of P in alfalfa tissue averaged 0.33 percent across all treatments. The Ca levels in alfalfa, however, were significantly higher in the hydrated plots than the unlimed or dolomitic lime plots while the concentration of Mg in the tissue was highest in the dolomitic plots.

For red clover, there were no significant differences in tissue nutrient levels between unlimed and dolomitic limestone plots (Table 52). The levels of P in the tissue treated with hydrated lime, however, was 42 precent lower than in the other lime treatments. At the single banded P treatment, the concentration of P in red clover tissue for unlimed, 2.3 t/ha hydrated and 13.8 t/ha dolomitic lime plots was 0.34, 0.19, and 0.35 percent, respectively. The levels of Ca in clover averaged 1.6 percent in the hydrated plots while the levels of the element averaged 1.0 percent in dolomitic plots. The Al concentration in the red clover did not vary between treatments, but the ratio of Al/P ranged from 0.03 in the unlimed plots and dolomitic plots to 0.05 in the hydrated plots.

Nodule number and weight per nodule increased with the rate of broadcast P in all the lime treatments for alfalfa (Table 53). With the high rate of dolomitic lime, nodule weight doubled with increasing broadcast treatments. The nodule number for alfalfa in the unlimed,

Table 51. Chemical composition of alfalfa from the Demerit experiment taken from the June 1979 harvest. Each value is the mean of four replications.

Lime treatment	Banded P	Broadcast P	N	Ca	Mg	P	K	Al
	KgP_2O_5/ha		%					ppm
0 t/ha	240	0	3.51 bcd*	1.03 a	0.22 ab	0.35 b	2.9 b	99 ab
	0	440	3.42 abcd	1.00 a	0.23 ab	0.31 ab	2.4 ab	121 cd
	120	220	3.56 cd	1.03 a	0.22 ab	0.31 ab	2.7 ab	107 abcd
	120	440	3.52 cd	1.07 a	0.22 ab	0.32 ab	2.5 ab	101 abc
	240	220	3.21 ab	1.07 a	0.22 ab	0.31 ab	2.8 b	121 cd
	240	440	3.50 abcd	1.07 a	0.22 ab	0.32 ab	2.5 ab	122 d
Hydrated Lime 1.0 t/ha	240	0	3.48 abcd	1.47 b	0.21 ab	0.23 a	2.6 ab	87 a
	0	440	3.41 abcd	1.56 b	0.23 ab	0.30 ab	2.8 b	107 abcd
	120	220	3.21 ab	1.61 b	0.20 a	0.32 ab	2.4 ab	107 abcd
	120	440	3.51 bcd	1.58 b	0.20 a	0.32 ab	2.2 a	100 abc
	240	220	3.56 cd	1.41 b	0.22 ab	0.34 b	2.5 ab	101 abc
	240	440	3.57 cd	1.43 b	0.22 ab	0.32 ab	2.5 ab	102 abcd
Dolomitic Lime 13.8 t/ha	240	0	3.42 abcd	1.03 a	0.26 ab	0.25 a	2.9 a	89 a
	0	240	3.27 abc	1.02 a	0.25 ab	0.32 ab	2.9 a	89 a

continued

Table 51 (continued)

Lime treatment	Banded P	Broadcast P	N	Ca	Mg	P	K	Al
	KgP_2O_5/ha		%					ppm
	120	220	3.20 a	1.02 a	0.27 b	0.32 ab	2.8 a	107 abcd
	120	440	3.61 d	1.03 a	0.26 ab	0.32 ab	2.5 ab	91 ab
	240	220	3.42 abcd	1.03 a	0.26 ab	0.35 b	2.5 ab	110 bcd
	240	440	3.43 abcd	1.03 a	0.27 b	0.32 ab	2.5 ab	121 cd

* numbers within a column followed by the same letter are not significantly different at the 0.05 level according to Duncan's New Multiple Range Law.

Table 52. Chemical composition of red clover from the Demerit experiment taken from the June 1979 harvest. Each value is the mean of four replications.

Lime treatment	Banded P	Broadcast P	N	Ca	Mg	P	K	Al
	KgP_2O_5/ha		%					ppm
0 t/ha	240	0	3.01 a*	1.01 a	0.25 ab	0.30 ab	1.9 ab	88 abcd
	0	440	3.02 a	1.04 a	0.25 ab	0.34 b	2.7 bcd	81 abc
	120	220	3.21 cd	1.06 a	0.28 abc	0.32 b	1.8 a	92 abcd
	120	440	3.06 abc	1.04 a	0.32 abcd	0.30 ab	2.2 abcd	89 abcd
	240	220	3.02 a	1.04 a	0.32 abcd	0.35 b	2.5 abcd	89 abcd
	250	440	3.03 ab	1.04 a	0.32 abcd	0.34 b	2.4 abcd	89 abcd
Hydrated Lime 1.0 t/ha	240	0	3.24 d	1.43 b	0.30 abcd	0.19 a	1.9 ab	91 abcd
	0	440	3.06 abc	1.67 bc	0.31 abcd	0.20 a	2.1 abd	97 bcde
	120	220	3.10 abcd	1.83 bc	0.23 a	0.20 a	2.3 abcd	91 abcd
	120	440	3.07 abc	1.86 c	0.30 abcd	0.20 a	2.8 cd	85 abcd
	240	220	3.21 cd	1.78 bc	0.31 abcd	0.19 a	2.5 abcd	91 abcd
	240	440	3.18 bcd	1.91 c	0.31 abcd	0.20 a	2.6 abcd	96 bcde

continued

Table 52 (continued)

Lime treatment	Banded P	Broadcast P	N	Ca	Mg	P	K	Al
	Kg P_2O_5/ha		%					ppm
Dolomitic Lime 13.8 t/ha	240	0	3.21 cd	1.11 a	0.38 cd	0.30 ab	2.0 abc	86 abcd
	0	440	3.01 a	1.00 a	0.39 d	0.32 b	2.6 abcd	110 de
	120	220	3.03 ab	1.00 a	0.40 d	0.35 b	3.0 d	71 cd
	120	440	3.04 ab	1.03 a	0.35 bcd	0.34 b	2.8 cd	67 a
	240	220	3.10 abcd	1.00 a	0.36 cd	0.34 b	2.8 cd	102 cde
	240	440	3.11 abcd	1.00 a	0.35 bcd	0.34 b	2.8 dc	121 e

* numbers within a column followed by the same letter are not significantly different at the 0.05 level according to Duncan's New Multiple Range Test.

Table 53. Nodule number and weight per nodule for alfalfa from the Demerit experiment. Each value is the mean of four replications.

Lime treatment	Broadcast rate	Nodule number per plant	Weight per nodule
	kg P_2O_5/ha		mg
unlimed	0	10 bc*	1.0 b
	220	18 d	1.4 b
	440	20 d	2.0 c
2.3 t/ha hydrated	0	4 a	0.5 a
	220	10 bc	1.2 b
	440	15 c	1.3 b
13.8 t/ha dolomitic	0	14 c	1.3 b
	220	21 d	2.4 c
	440	22 d	5.1 d

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

2.3 t/ha hydrated and 13.8 t/ha dolomitic lime at 440 kg broadcast P_2O_5/ha was 20, 15, and 22, respectively; and the weight per nodule over the same treatments was 2.0, 1.3, and 5.1 mg, respectively. The hydrated lime seemed to inhibit nodulation.

For red clover, nodule number and weight also increased with the rates of broadcast P, but the magnitude was smaller than for alfalfa (Table 54). In the unlimed plots, the increase in nodule number due to broadcast P for alfalfa and red clover was 50 and 20 percent, respectively. For red clover, the weight per nodule never exceeded 1.4 mg while for alfalfa, the nodules weighed between 0.5 to 5.1 mg. Hydrated lime decreased nodulation in red clover, reducing nodule number by 50 percent compared to the dolomitic lime plots.

The data in this experiment indicated that the broadcast P reduced Al saturation and increased the available P levels in the unlimed plots such that the yields of the two legumes in these plots were comparable to the dolomitic lime plots. The adverse influence of hydrated lime on nodulation of alfalfa and red clover is probably due to an interaction between soluble P and the lime:

$$Ca^{++} + \underset{\text{soluble}}{Ca(H_2PO_4)_2} \rightarrow \underset{\text{insoluble}}{Ca_3(PO_4)_2} + H^+$$

According to Norris (1965), high levels of soluble Ca are especially detrimental to clovers tolerant to soil acidity. Norris stated that Rhizobium and host plant which have evolved in acid soils have developed an intolerance to alkaline conditions.

Table 54. Nodule number and weight per nodule for red clover from the Demerit experiment. Each value is the mean of four replications.

Lime treatment	Broadcast rate	Nodule number per plant	Weight per nodule
	kg P_2O_5/ha		mg
unlimed	0	39 c*	0.6 ab
	220	49 de	1.0 bc
	440	53 ef	1.3 c
2.3 t/ha hydrated	0	10 a	0.3 a
	220	19 ab	0.3 a
	440	29 b	0.5 ab
13.8 t/ha dolomitic	0	48 cd	0.8 b
	220	63 f	1.2 c
	440	59 f	1.4 c

*Means within a column followed by the same letter are not significantly different at the 0.05 percent level according to Duncan's New Multiple Range Test.

IV. SUMMARY AND CONCLUSIONS

Alfalfa nodulation, nitrogen fixation, and yield were more highly correlated to Al saturation than pH in Paxton, Agawam, and Charlton soils. At the highest limestone treatment, the Al saturation was reduced to less than 1 percent, and there were no differences in alfalfa growth. Even though the added Ca likely benefited nodulation, the major benefit to alfalfa growth was the reduction of Al. Lime coating had no influence on red clover, but increased nodule number and weight for alfalfa without benefitting yield or nitrogen fixation. Results from the ^{32}P experiment showed that red clover was capable of mobilizing P in the presence of Al to a far greater degree than alfalfa, and this ability was reflected in the lower Al/P ratios in clover nodules compared to alfalfa nodules.

The addition of lime to Paxton soil reduced Al saturation significantly but had no influence on available P. The superphosphate experiment showed that once the Al saturation is reduced, the application of P had far greater influence on alfalfa growth than dolomitic or hydrated lime. These experiments on soil acidity, liming and P demonstrate that alfalfa growth is dependent on the reduction of Al saturation by liming, the addition of which supplies Ca required for nodulation. After Al reduction, the primary importance then is not the increase of soil pH to 6.5 but the improved availability of P for dry matter accumulation which is highly correlated to substrate supply for nitrogen fixation.

The aeroponic study examined the growth of alfalfa and red clover at pH 5.0 with 0, 1, 2 ppm Al and 2 ppm + N. Results showed that at these levels of Al, clover experienced a maximum reduction in fixation of 16 percent at 2 ppm; under the same conditions, the reduction in fixation for alfalfa exceeded 70 percent. Upon the addition of combined N, nitrogen fixation was reduced in both legumes by 90 percent, but the yields were not significantly different from the plants at 0 ppm Al. Results from the addition of combined N indicated that the Al influence on legumes was principally on the symbiosis.

The influence of N on nitrogen fixation in alfalfa and red clover was further investigated in a soil experiment using 0 and 60 kg N/ha. The applied N increased nitrate reduction with a compensating decrease in nitrogen fixation such that yields were not affected. The Km for nitrogenase is 0.03 to 0.05 atm (Bergensen, 1977) which indicates that the partial pressure of N_2 is not the limiting factor in nitrogen fixation. Hardy and Havelka (1976) found that increasing the level of CO_2 from 300 to 1000 ppm caused exponential increases in nitrogen fixation, a doubling of yield, and a severe reduction in nitrate assimilation for soybeans. The data from Hardy and Havelka indicated that the limiting factors for nitrogen fixation and nitrate assimilation are substrate supply and soil N, respectively. The probable reason for the lack of response to the added N fertilizer is that N is not the limiting factor for yield; through nitrogen fixation, the plants have an unlimited N supply.

The adverse influence of Al is not limited to the symbiosis but also affects the growth of the <u>Rhizobium</u>. In culture, the addition of

Al to the media at pH 5.0 severely reduced the growth of R. meliloti. At the higher pH's, the effects of the Al could not be separated from the possible formation of Al-P complexes in the media. The differential tolerance to the R. meliloti strains to Al and pH in culture did not correspond to the results in the soil. Even though culture experiments may be useful in selecting Rhizobium strains for tolerance to Al, there is no guarantee that the strains will interact with a plant under field conditions to improve growth.

Amending the soil with lime and P not only improves the growth of the legume but influences the growth of the Rhizobium in the soil. Alfalfa and red clover seeded into soil from the Demerit experimental site showed that applied P had the most significant influence on nodulation for both inoculated and inoculated plants. In the Wallman soil, the most important factor in legume nodulation was the length of time the soil had contained legumes before collection. These experiments indicated that the encouragement of soil populations of Rhizobium requires the reduction of Al saturation, improved availability of P and the presence of the host plant.

The application of broadcast lime to the soil one year prior to seeding reduced Al saturation from 16 to 1 percent in the top 5 cm of the soil profile which enhanced legume yields. The banded lime had no value in terms of yield, percent stand, or persistence of alfalfa or red clover. The influence of low P levels and high Al saturation in the deeper regions of this soil can only be determined over time in terms of stand persistence. The P applied at the surface of this soil moved into the profile slower than the lime so that effecting a significant increase in available P in the deeper profile may be quite difficult.

At the Demerit site, the initial pH was 5.6 with an Al saturation of 2 percent in the top 5 cm of the soil profile. Hydrated lime increased the pH to 6.5 and reduced Al saturation to 0 percent one month after lime application. But the use of this material gave no yield benefit to alfalfa and reduced nodulation in both legumes. The single application of banded P and the combination of the high rates of banded and broadcast P gave alfalfa yields in the unlimed plots equal to those in the hydrated and dolomitic lime plots. The data clearly shows that P application in this soil was more of an advantage than liming.

In conclusion, the usual policy of increasing soil pH to 6.5 for optimal legume growth is not as critical as reducing Al saturation to 5 percent and increasing available P. The pH at which this occurs varies between soils, but in the Paxton silt loam and the Hinckley sandy loam, pH's of 5.6 to 5.8 were adequate. The depth to which these changes in soil chemistry are critical to legumes is not entirely clear, but the present data indicated that reduced Al and increased P in the top 10 cm of the soil profile were sufficient for first and second year legume growth.

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